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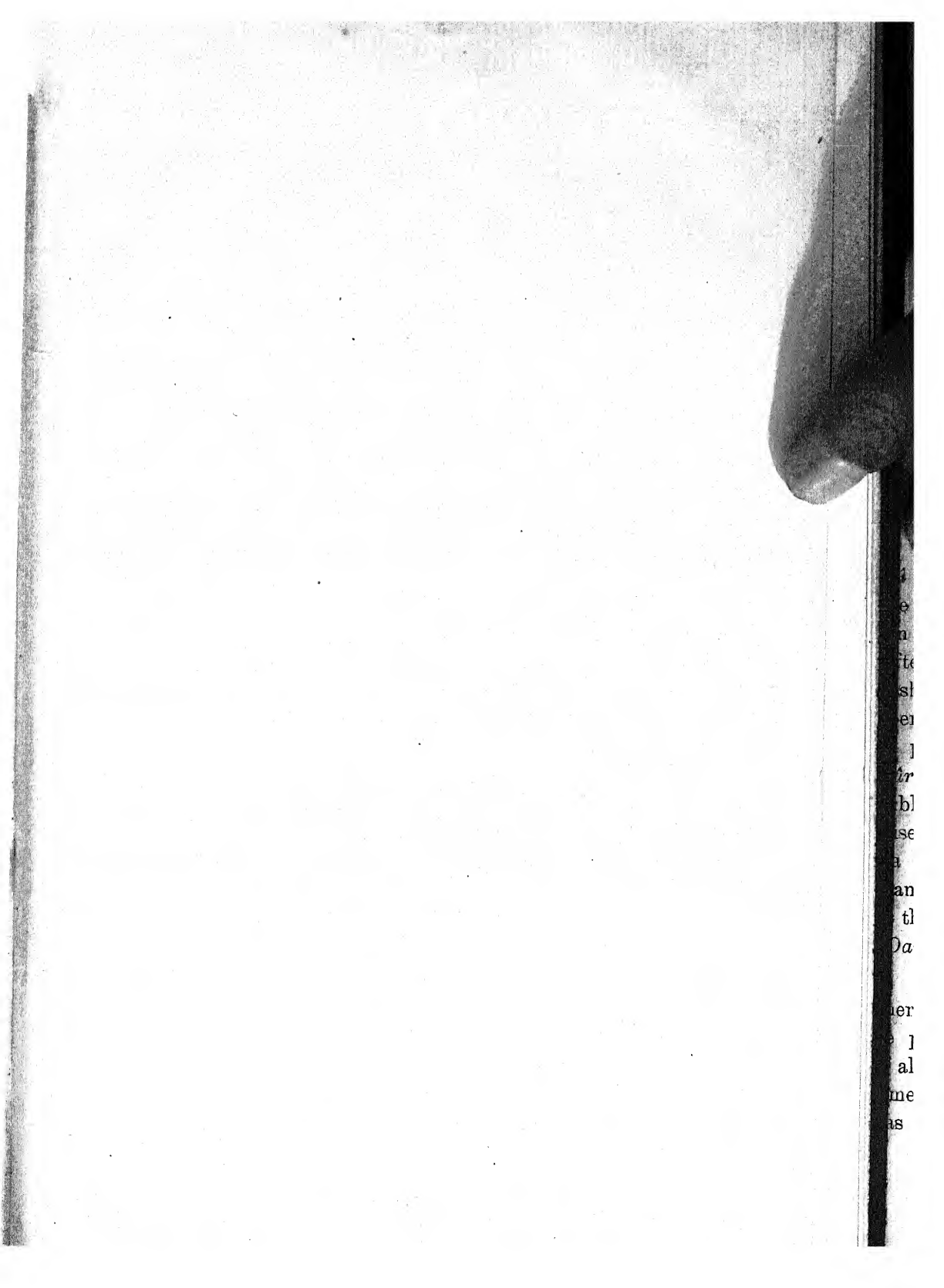
INDEX OF SUBJECTS

INDEX OF AUTHORS



THE

NEW PHYTOLOGIST

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THE FUNDAMENTAL FAT METABOLISM OF THE PLANT

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CONTENTS

INTRODUCTION

WRITTEN as the introduction to a series of investigations chiefly concerned with the metabolism of fat in the plant[1], the following paper contains the statement, as simple and full as possible, of the chemical and physiological side of the problem, in the hope that chemists and botanists may help towards a solution. Valuable criticisms have been already received, and a part embodied in the paper, and the writer must especially acknowledge the help received from Prof. H. S. Raper of the Department of Physiology, the University of Manchester, and from Mr H. D. Kay, Beit Research Fellow, both formerly on the staff of the Department of Physiology, University of Leeds. The chemical side of the work owes a great deal to the valuable assistance given by Mr R. M. Woodman, M.Sc., whilst a research scholar of the University, and while the writer is prepared to take full responsibility for the general views advanced, he wishes to acknowledge as fully as possible his indebtedness to Mr Woodman

[1] This paper was written before the paper upon "The Composition of the Cell-Wall at the Apical Meristem of Stem and Root," by R. M. Tupper-Carey and J. H. Priestley (*Proc. Roy. Soc.* 95 B, pp. 109–131, 1923), in which some of the results of these investigations are published.

for many of the chemical data employed to support hypotheses put forward.

As Osborne (20) has recently pointed out in the case of proteins, our knowledge of certain classes of organic compounds, of primary importance in plant physiology, is liable to be misleading because based entirely upon the study of storage products. The formation and use by the plant of temporary fat reserves is described in such recent text books as Haas and Hill (7), Onslow (19), etc., and in recent monographs such as those by Terroine (36, 37), Leathes (11), etc. This problem is not discussed further here. But Terroine (37) in his recent account of the metabolism of fats in the animal organism draws a distinction between the metabolism of fat reserves and that metabolism of fat which is inseparable from growth. It will be the object of the following pages to review the data available as to fat metabolism within the plant in the effort to establish the existence of a similar metabolism which is inseparable from the growth of the living plant. It is in this sense, and in contradistinction to the metabolism associated with the formation and utilization of temporary reserves of fat, that the phrase "fundamental fat metabolism" is employed to denote that metabolism of fatty substance which must be one of the fundamental conditions associated with growth. In the next section we are concerned with definitions of the chemical categories of the substances concerned.

FATS AND LIPINS

If the botanist is to attain to an understanding of the complex phenomena associated with the living plant he must be prepared to make acquaintance with a very wide range of chemical compounds, characterized by the organic chemist. Botanical texts have so far not devoted much attention to the group of fatty substances and in recent botanical papers there is much use of the word "lipoid," which is best used as it was first employed by Overton (21), namely, as a general term for all substances dissolving in the usual fatty solvents, many of which are chemically not related to the fats at all. To follow further the rôle of fats within the plant it is essential to grapple with the chemical characteristics of the different types of compound involved. In this section the attempt is made to define these classes and to explain in as elementary a way as possible their main chemical characteristics; references are supplied to monographs which provide essential fuller data but from which the botanical reader is perhaps repelled for lack of a simple enough introduction to their

chemical complexities. At the risk of crudities of statement, the attempt is made to provide this introductory simplification because unless botanists generally will attempt an understanding of the chemical phenomena lying at the base of metabolism and of structural and morphological problems also, there seems a danger of a real entity—Botany—being lost to sight amidst the labours of specialists working in the fields of plant physiology, biochemistry, anatomy, etc. Because the attempt is made to present a simple statement of the problems involved in fat metabolism it is not intended to convey the impression that the phenomena are simple or their investigation easy; on the contrary, some of the groups of compounds to be mentioned offer very great difficulties in interpretation, particularly because the technique of their isolation and investigation is so difficult and laborious and as a result many contradictory data have accumulated.

Fat. The word "fat" has first to be explained, because apart from its general use in every-day speech, it is employed with a strict chemical meaning.

No organic compounds are better known than the alcohols, such as ethyl alcohol, C_2H_5OH, and the acids, such as acetic acid, CH_3COOH, which owe their characteristic chemical properties mainly to the hydroxyl (—OH) and carboxyl (—COOH) groups respectively. These substances under many conditions tend to unite by their —COOH and —OH groups, with the elimination of water, thus:

$$—CO\boxed{OH + H}O—;$$

acetic acid and ethyl alcohol in this manner combine to form ethyl acetate, $C_2H_5O—OCCH_3$. In this compound, a typical "ester," $C_2H_5O—$ is the basal group of the alcohol, whilst the characteristic basal group, or radicle, of the acid, $—OC.CH_3$ is known as the "acyl" group. In the living organism, a very common alcohol is glycerol,

$$\begin{array}{l} CH_2OH \\ | \\ CH.OH \\ | \\ CH_2OH \end{array}$$

a trihydric alcohol, *i.e.* containing three hydroxyl groups, and all or some of these can combine with carboxyl groups thus linking glycerol to the acyl groups of one or more acids.

Usually the acid so linked on in the organism is a very complex one, in which the $CH_3—$ of acetic acid is replaced by a long chain of carbon atoms, *e.g.* $CH_3—CH_2—CH_2—CH_2—$, etc.

Such acids are known as *fatty acids*, and chemically fats are esters of glycerol, in which one or more of the hydroxyl groups have combined with the acyl groups of fatty acids with the elimination of water. If we write the symbol R for the long carbon chain and its termination —CO of the acyl group of the fatty acid, then we may represent a completely combined glycerol ester, or tri-glyceride as follows:

$$\begin{array}{l} CH_2O\ R_1 \\ | \\ CH\ O\ R_2 \\ | \\ CH_2O\ R_3 \end{array}$$

where R_1, R_2 and R_3 may be radicles of the same fatty acid or of three different fatty acids.

In a natural fat, R_1, R_2 and R_3 attached to the same molecule of glycerol are usually identical, but in any such fat different molecules of glycerol are always found to be combined with more than one kind of fatty acid, so that all natural fats may be described as esters, or glycerides, of a mixture of fatty acids. This is the strict technical use of the word "fat"; if the substance when isolated is liquid at the ordinary temperature it may be termed an oil, but "oil" is a word that is used still more loosely and in chemical composition such an oil is still a "fat." Frequently the fatty acids present in the glycerol ester have two of their carbon atoms linked thus,

$$\begin{array}{l} —C=C— \\ \ \ H\ H \end{array},$$

by a double bond: such fatty acids are described as unsaturated, because they have a tendency to pick up more hydrogen, so that this linkage becomes

$$\begin{array}{l} —C—C— \\ \ \ \|\ \ \| \\ \ H_2\ H_2 \end{array},$$

but they will readily combine with oxygen at this linkage, whilst the fact that iodine is picked up in the same way, giving

$$\begin{array}{l} \ \ I\ \ I \\ \ \ |\ \ | \\ —C—C— \\ \ \ |\ \ | \\ \ \ H\ H \end{array},$$

provides a means of measuring the degree of unsaturation of the fatty acid or fat. This is expressed by the "Iodine Number," *i.e.* the number of grams of iodine with which 100 grams of the fat combine under standard conditions (Leathes(11)).

An unsaturated fatty acid is usually liquid at ordinary temperatures whilst the natural saturated fatty acids are usually solid and the acids appear to transmit this characteristic to their respective

glycerides. The result is that vegetable oils are usually more unsaturated than vegetable fats.

The fats fall therefore into two groups when arranged according to their iodine numbers: (1) the "oils" or liquid fats; (2) the "fats" or solid fats. The oils are characterized by higher iodine numbers.

Occasionally, more especially in surface layers such as the cuticle, we may find fatty acids combined with the higher monohydric alcohols. The ester is then technically termed a wax. The name "wax" does not denote any special physical wax-like properties. In both secondary endodermis and cuticle evidence has been found of the frequent presence of one type of higher alcohol, phytosterol. These alcohols, allied to the cholesterols of the animal kingdom, contain besides their hydroxyl group, one unsaturated linkage; they are probably as widespread throughout the plant kingdom as cholesterol throughout the animal.

Lipins. Fatty acids are also found in combination in substances known as lipins (Maclean (14)), which are divisible into two groups:

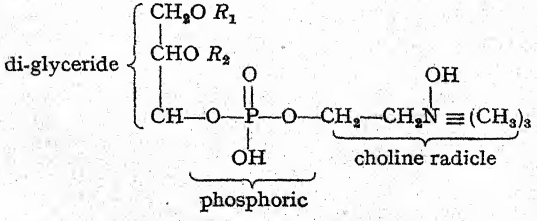
(*a*) phosphatides, containing fatty acids, phosphorus and nitrogen;

(*b*) cerebrosides, containing fatty acids, nitrogen and a sugar group, *but no phosphorus.*

Phosphatides. The only three phosphatides known at present are lecithin, cephalin and sphingomyelin. Lecithin and cephalin are yellowish-white waxy substances of a hygroscopic nature. When dried *in vacuo* they are soluble with difficulty in hot alcohol, though readily soluble when moist.

Lecithin may be regarded as glycerol in which two hydroxyl groups have contracted the usual linkage with the carboxyl groups of a fatty acid whilst the third hydroxyl group has combined with one hydrogen atom in phosphoric acid and been eliminated as water, leaving the phosphoric acid combined on the one side with the di-glyceride, while on the other a hydrogen atom is replaced by the radicle of the nitrogen-containing base, choline.

Thus lecithin may be written



R_1 and R_2 represent the acyl groups of different fatty acids.

From pure lecithin only one base, choline, has been isolated. The fatty acids yielded on hydrolysis of egg-yolk and brain lecithin are identical and consist of one unsaturated acid, oleic, and two saturated acids, palmitic and stearic. This lecithin appears therefore to contain *three* acyl groups. Liver lecithin, on the other hand, yields two unsaturated acids, either two of the linoleic series or one of the linoleic and one of the oleic series, and two saturated acids, stearic and palmitic. Liver lecithin therefore contains four acyl groups. But the formula given above for lecithin provides only for two acyl groups. This problem is fully discussed by Levene (12); the evidence appears to favour the retention of the simple monophosphatide formula for lecithin and the assumption that there are usually mixtures of such lecithins present in natural products, these differing simply in the nature of the fatty acids present.

Cephalin is built on the same lines and differs from lecithin principally in containing a different base, β aminoethanol (or "colamine") in place of choline. The acids so far isolated from the hydrolysis of cephalin are stearic and linoleic.

It should be noted that no pure phosphatides have probably so far been isolated from the plant. Winterstein (41) has shown that the lecithin extracted from plants is usually associated with up to 16 p.c. of carbohydrate (glucose, galactose, pentose and methyl pentose). These carbohydrates are possibly present in combination as they cannot be entirely removed by constant washing with water and alcohol.

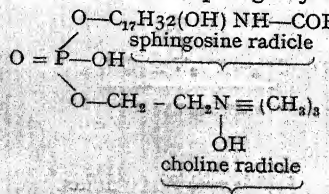
Sphingomyelin contains no glycerol radicle. The products of hydrolysis are phosphoric acid, fatty acids and two bases, choline and sphingosine, all in equivalent proportions. The ratio of N : P is therefore 2 : 1 and not 1 : 1 as in lecithin and cephalin.

On fractionally distilling the methyl ester of the isolated organic acids two fractions are obtained in equimolecular proportions, viz.:

First fraction: methyl ester of lignoceric acid.

Second fraction: methyl ester of an acid of lower carbon content (probably hydroxystearic acid).

To give the simplest possible formula for sphingomyelin, therefore, we must assume *two* sphingomyelins in which the acyl radicle differs:



(R = in one case lignoceric acid, in the other possibly hydroxystearic)

Sphingosine has never so far been isolated from the plant, but this negative evidence cannot be considered as conclusive proof of the absence of sphingomyelin, whilst there is always the possibility of other phosphatides with similar N : P ratios although without sphingosine in the molecule.

Cerebrosides. The second type of lipin, the *cerebrosides*, may also be regarded as galactosides: they owe their name to their presence in animal brain tissue. They are substances containing the hexose galactose in combination in equi-molecular proportion with a fatty acid and sphingosine. Two cerebrosides are definitely known, phrenosin and kerasin: the former on hydrolysis yields lignoceric (formerly termed phrenosinic) acid, the latter yields kerasinic acid.

Again, the absence of sphingosine in the products of hydrolysis of plant materials cannot definitely decide against the presence of cerebrosides in the plant. Too little work has been done in this direction as yet to give much significance to negative results.

The frequent occurrence of galactose and galactosans (*i.e.* anhydrides, condensation products of galactose) in plant tissues and the fact that Trier(38), from extractions of cereal grains has obtained lipin-like products with a high N : P ratio, leads one to think with Trier(38) (*loc. cit.* p. 107) that the possibility of the occurrence of compounds of this type requires further investigation. It is always possible that similar cerebroside-like linkages may be found with another base replacing sphingosine. Some of the work carried out in the writer's laboratory with the seeds of *Vicia Faba* L., the broad bean, has again awakened suspicion that cerebrosides may be present in these tissues. Mr Woodman has therefore faithfully followed Rosenheim's pyridine method, as applied to brain tissue(30): he has also on another occasion first extracted large quantities of broad bean meal with alcohol at 50–60° C. and subjected the residue obtained on concentrating and cooling the extract to extraction by the pyridine method.

Negative results were obtained, no base being identified in the very small yield of products obtained. These results are only recorded for information, however, and not in any sense as conclusive evidence for the absence of cerebrosides within the plants.

The separation and isolation of the fatty acid-containing bodies from the plant, necessitates very large supplies of raw materials and of organic solvents and considerable experimental manipulation. Probably these factors account to some extent for the slow progress made towards an understanding of the metabolism of fatty substances in the plant. Further Terroine(36) has recently shown con-

vincingly how very little reliance can be placed upon many of the data so far contributed towards a solution of the problem. Future progress is also likely to be slow and dependent upon the gradual establishment of a reliable technique; but no problem of plant metabolism more urgently demands investigation.

DISTRIBUTION OF FATTY SUBSTANCES IN THE PLANT

The microchemical data as to the distribution of fatty substances in the plant are so far vague and untrustworthy; but the useful summary of technique given by Cramer in the new edition of Bolles Lee ((3), *loc. cit.* pp. 356–368) should accelerate the accumulation of fresh and more reliable facts. In discussing the question as to the distribution of fatty acids and their compounds in the plant we must distinguish between the occasional large accumulation of temporary fatty reserves in certain tissues and the frequent, possibly invariable, occurrence of smaller quantities of fatty substances in different parts of practically every plant at all seasons of the year. The following paragraphs represent an attempt to summarize the available knowledge as to the general distribution of fatty substances in the tissues of the vascular plant irrespective of the temporary accumulation of reserves of fat.

1. The apical meristem, the actively growing apical region of a vascular plant, where the main bulk of plant protoplasm seems frequently to be constructed, is probably the most active centre of protein synthesis in the plant. It appears always to contain relatively large quantities of fatty substances, notably fats and phosphatides. The microchemical survey of this tissue, directed to the detection of these fatty substances, has not up to the present been very extensive[1]. Czapek(5) gives a special technique for the detection of "lipoids" in such a tissue. Recognizing that the difficulty of detection is largely due to the fine state of dispersion of the "lipoid" in the dense substance of the protoplasm, he introduces into the cell the fat solvent pyridine in solution in tertiary amyl alcohol. The finely dispersed "lipoid" is thus collected into drops which are stained by the usual fat stain, Sudan III, dissolved in the mixed organic solvents. The writer has tested this method and it certainly demonstrates the

[1] See, however, the data given in paper cited in footnote on p. 1. No attempt is made to discuss the chemical nature of organized cytoplasmic structures such as the chondriosomes, described by L. W. Sharp (*Introduction to Cytology*, New York, 1921, *loc. cit.* p. 117), as containing phosphatide and albumin, because the chemical natures of all these structures is still very obscure (see A. Meyer, *Analyse der Zelle*, 1, Jena, 1920).

presence of fats in thin sections of plant growing points. A simpler method gives, perhaps, a clearer indication of the presence of these lipoids, possibly releasing them from various forms of combination with the proteins predominantly present in such a cell. Sections of plant growing points are soaked in alkaline potassium hypochlorite solution ("Eau de Javelle," Molisch (17)) for 24 hours or longer, when the proteins are largely decomposed and dissolved, but in practically every cell of the meristem large globules or granules of substance are left. These dissolve in the usual fat solvents and may therefore well be described as "lipoids," a term which is still useful if used in the strictly noncommittal sense introduced by Overton (21) for substances which dissolve readily in fatty solvents. These globules are peculiarly soluble in warm alcohol, they have been examined to some extent upon a macro-chemical scale, powdered dry radicle of broad beans, after previous extraction with hot ether and alcohol, being left for 48 hours with alkaline hypochlorite, then dried and re-extracted with hot ether and alcohol. The residue from the alcohol extraction, appears to consist mainly of phosphatide; and, in the presence of water, the residue, either from the dried radicle or dried plumule of the broad bean, shows magnificent "myelin" forms under the microscope.

2. Fats, fatty acids or soaps can be detected in the walls of plant cells from a very early stage and appear to be present as early as the cellulose and pectic substances regarded as typical of the plant wall. The presence of these fatty substances causes the difficulty in obtaining the cellulose reaction from many parenchymatous tissues with the chloriodide of zinc reagent unless the sections are previously warmed with dilute potash. Hansteen-Cranner (8, 9, 10) has given considerable experimental evidence of the presence of fats and phosphatides in the membrane of normal parenchymatous tissues and has pointed out the importance of this conclusion in relation to the behaviour of such tissues in the presence of various cations, some of which, *e.g.* Ca, form insoluble soaps with fatty acids whilst others, *e.g.* K, Na and Mg, form soaps soluble in water.

3. Behind the growing apex of the plant all tissues are at first parenchymatous. Some of the cells retain their cellulose walls throughout life, others differentiate, the vascular strand being formed internally and various types of protective tissue at or near to the surface. Of the vascular tissues, the phloem, although sometimes fat is included in the cell contents, has walls which are remarkably free from fat. This is probably due to the relatively alkaline reaction of the sap of the phloem, a phenomenon noticed long ago by Sachs (31),

and which may result in the complete removal of fatty acids from the walls in the form of soluble potassium or sodium soaps. The walls of the phloem and of the adjacent sclerenchyma are the only walls of a typical Angiosperm stem which are usually found to give a blue reaction immediately upon treatment with chloriodide of zinc. The xylem vessels and tracheids have completely lost all their protoplasmic contents including most of the fatty substances, but to judge from their staining reaction traces of these fats may still be present in the membrane of these conducting elements.

4. The membranes of all the external protective tissues of the plant, which are less readily permeable to water, in every case appear to owe this property to the presence of oxidation and condensation products of fatty acids. These products, usually known in structural botany as suberin or cutin, terms which certainly cover a large variety of chemical aggregates, give rise upon saponification to oxy-fatty acids, *i.e.* fatty acids in which hydroxyl groups are present, the "suberogenic" or "cutinogenic" acids, of varying composition and present in varying proportions (Priestley (23)). Oxidation and condensation products of fatty acids, especially unsaturated fatty acids, also go far to give to the endodermis the very characteristic and important physiological properties it possesses (Priestley and North (25), Priestley (22)).

5. In the assimilating tissues, fats and phosphatides are always present, and to judge from the evidence as to their abundance in green and etiolated plants (Maclean (14), *loc. cit.* p. 167), these fatty substances are probably synthesized by green plants in the light. The pigments of the green leaf are probably dissolved in colloidal solution in lipoids (Stern (34)) and there are many instances given in the literature in which the plastid containing the pigments, the chloroplast, ceases to form starch or any detectable carbohydrate and on the other hand commences to accumulate fatty substances. Such plastids are then sometimes termed elaioplasts (Beer (2)).

The conditions under which starch or carbohydrate formation is replaced by synthesis of fat or oil are still very obscure. Some observations (Tuttle (39), Lewis and Tuttle (13)) would suggest that temperature changes might produce this effect. Probably of greater significance is the observation, which the writer owes to Dr Pearsall that the green algæ in which carbohydrate formation is replaced by fat formation are characteristic of waters in which the concentration of salts is high (as salt water) or in which the ratio of Ca, Mg, to K, Na is unusually high.

The above paragraphs include all the salient features of fat distribution, irrespective of local food reserves, which seem to the writer to be well established as the result of investigation to date. The extent and precision of these data should be rapidly increased in the next few years. As the method of investigation must be mainly micro-chemical, there are two comments on micro-chemical methods as used at present that seem desirable in the light of the writer's experience of them.

Nile blue has apparently proved a valuable reagent in animal histology because of its capacity for distinguishing between fatty acids, with which it forms a blue compound, and neutral fats, which it colours pink (Cramer (3)). In the plant Nile blue has proved of great value for identifying neutral fat, but its blue reaction must be regarded as in itself no indication of the presence of fatty acid since experience of the reaction has demonstrated beyond a doubt that it will give the same reaction with pectic acid.

All the micro-chemical reactions which are said to be characteristic of fat appear to be due in some way to the unsaturated linkage present in the fat (Mann (16), Cramer in Bolles Lee (3)), although the actual mechanism of the staining reaction appears in nearly every case to be obscure. As the fats and fatty acids present in the plant seem invariably to include a certain proportion of unsaturated acid this may not be a practical difficulty, but the point is of considerable importance and micro-chemical methods could be employed with greater certainty if there were fuller knowledge of the physical chemistry of the reactions involved. Mangin's use of ruthenium red (15) as a stain for pectic acid is a case in point. The similarity of behaviour of compounds of ruthenium to those of osmium, suggests that the presence of an unsaturated linkage or other reducing agent might produce the deposit of the ruthenium in the tissue, and the experience gained with ruthenium red in the laboratory has led the writer to regard it as unreliable as a reagent for pectic acid when fatty acids may be present.

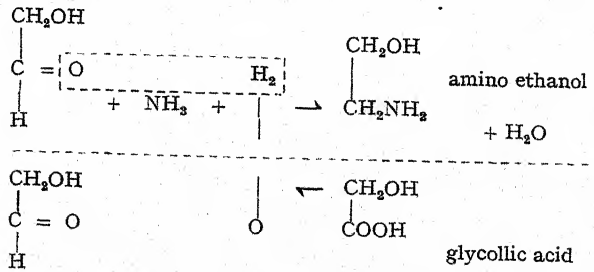
Synthesis of Fatty Substances at the Photosynthetic Centres

As already indicated, there is evidence from the quantity of fatty substances obtained in etiolated plants as compared with plants grown in the light, that these fatty substances are synthesized in the light. The fat soluble vitamin which is present in plant tissues appears similarly to be formed only in the light (Coward and Drummond (4)).

Unfortunately in the present state of our knowledge of photosynthetic metabolism it is not likely that much light can be thrown upon the course of chemical events during synthesis of fatty acids and fatty substances in conjunction with the synthesis of carbohydrates. At present the physiological evidence as to the synthesis of fats in the plant suggests that the synthesis starts from carbohydrates (Neuberg and Arinstein (18) and Smedley and Lubrzynska (33)). As lipins appear to be formed at the same time brief consideration may be given to the problem of the source of the other constituents necessary for their construction.

The presence of glycerol at a photosynthetic centre offers no great difficulty, for it is very closely allied to a triose sugar. Phosphoric acid arrives from the soil, but at first sight the existence of the base is not so readily explained. The necessary nitrogen is certainly present in the leaf as a shifting equilibrium of nitrate and nitrite (Schimper (32), Baudisch (1), etc.); but in the presence of sugars and in an alkaline medium, doubtless provided in the stroma of the chloroplast, where carbonic acid is continually disappearing in the sunlight and where potassium is always strongly represented, the presence of nitrite would certainly involve the formation of ammonia (Baudisch (1)).

Glycolaldehyde, present as a result of photosynthesis, may react with ammonia to give amino-ethanol, the base present in cephalin, reduction taking place simultaneously.

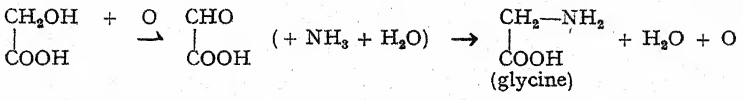


This reaction would be analogous, to a certain extent, to the well-known reaction occurring in the body, in which keto-acids react with ammonia to give amino-acids and amino-acids are oxidized to give keto-acids.

$$R.CHNH_2—COOH\ (+\ O) \rightleftharpoons R.CO—COOH + NH_3.$$

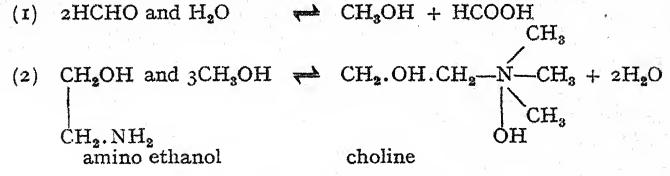
The glycollic acid produced may be oxidized to give glyoxalic acid, which, by the action of ammonia might undergo a similar reaction

to the one above (simultaneous reduction taking place), giving glycine, a basal substance in protein metabolism.



This suggestion as to phosphatide synthesis is, of course, hypothetical. A consideration of the scheme of reactions suggested shows that the synthesis of phosphatides is probably only dependent on light because light is necessary for the original carbohydrate synthesis. Apart from this original carbohydrate synthesis from carbon dioxide probably no external energy is needed for this series of reactions. It is therefore conceivable, as Suzuki (35) and Zaleski (42) have shown for proteins, that the synthesis of phosphatides might be expected to take place in the dark also if the centres of synthesis were adequately supplied with carbohydrate and with nitrogen in some appropriate form of combination.

Finally an indication may be ventured as to how the amino-ethanol contained in the cephalin molecule is converted into choline, the lecithin base. This change involves methylation, which is a biochemical process of frequent occurrence in the plant and urgently requiring investigation. The only suggestion that has been made is that formaldehyde undergoes the Canizzarro transformation, giving rise to methyl alcohol; this methylates the amino-ethanol of the cephalin molecule, giving lecithin (Trier (38), *loc. cit.* pp. 47, 48).



It is, however, a significant fact that formaldehyde itself may be regarded as a methylating agent; its use to this end has led Robinson (29) to his suggestions for the synthesis of alkaloids in the plant.

Formation of Fatty Substances in the Meristem

The fact that new fatty substances are apparently only formed in the shoot of the germinating seedling when the leaves reach the light, seems to have caused an implicit assumption to be made by every author that the fats of the meristem have been supplied to it

by translocation from other parts of the plant. If the meristem of the growing point be provided with carbohydrate there would seem to be no *a priori* reason why it should not manufacture its own fatty substances. In the present state of our knowledge no definite statement on such a problem is possible; the remainder of this section will be devoted to a brief statement of the reasons which cause the writer to entertain, as the most probable hypothesis, the view that the fats of the meristem are formed as a result of the metabolic activity of the cells of the meristem.

(1) A careful study of the distribution of fatty substances in the meristem and in the tissue differentiating from it has shown a progressive change in the distribution of these fatty substances. Appearing first of all in a very fine state of dispersion throughout the protoplasm of the cell, they appear to collect into small droplets in the peripheral region and finally to reach the surface layer of the protoplast; from thence they pass on to the wall surrounding the protoplast. Apparently first of all held chemically by the wall, then released from chemical union as fatty acids, they diffuse along the walls until precipitated as insoluble salts, especially in the middle lamella, or until precipitated in altered forms at the surface of the plant or in special layers such as the endodermis. This part of the subject is dealt with further in the next section. These facts as to distribution do not argue in favour of a passage of the fats from sap originally outside the cell into the interior of the cell, but by themselves they could not be given much weight.

(2) The fatty substances of the meristem include lipins. It is unlikely that complicated fatty compounds of this kind are brought to the apical meristems by the nutrient sap, although fatty acids or soluble fatty soaps might move in this manner, so that in any case we have to consider the synthesis of some of these fat compounds as occurring in the meristem cell.

As their subsequent hydrolysis takes place during differentiation, the facts certainly suggest that lipase must be present in the cell at this stage, lipase apparently being capable of hydrolysing lipins as well as fat, though not cerebrosides. If lipase is present at this stage then presumably it may be active as a catalyst in synthesis when conditions in the growing meristem are favouring condensation. Such conditions, essential to synthesis, undoubtedly exist in the non-vacuolated cells of the meristem.

(3) If fats or fatty acids have to be supplied to the growing meristem; in view of the quantity present in a meristem (4·5 per cent.

of dry weight in the meristem of the root of the broad bean) it should be possible to detect the fats moving through the tissue of the plant. Every spring the sap supply from the roots rises into the stems and quickens into vigorous activity the meristems of leaf and stem. This sap can readily be collected and under superficial examination shows the presence of slight traces of a substance soluble in fatty solvents and of very appreciable quantities (more than 1 per cent.) of a carbohydrate. No organic nitrogen can be detected in the liquid but nitrites are present (Priestley and Armstead (24)).

It is very difficult to see how the minute supplies of unidentifiable lipoid substance (which are possibly not chemically allied to fats) in this liquid can maintain the supply of fat in the meristems it supplies; but still more clear is it that nitrogen synthesis appears to be commenced *de novo* with inorganic nitrogen. Phosphates are present in the liquid as well and the conclusion seems irresistible that when these carbohydrates and nitrites reach the meristem a series of linked reactions is initiated such as is described in the previous section and fat and lipins appear, in a sense as by-products, in the synthesis of protein and protoplasm. Essential by-products in all probability, because the writer is forced to regard the rôle played by these lipoid substances as they pass from the general ground substance of the cell to its periphery and then out into the surrounding wall as fundamental in relation to the varying permeability of the cell, from the time it forms part of the meristem to the time when it vacuolates and differentiates behind the growing apex. Levene (12) has pointed out that, like proteins, the phosphatides are amphoteric and should help to maintain the surface charge of the protoplast membrane.

(4) In one case that has long been under examination in the laboratory, with the aid of Miss L. M. Woffenden (26), the writer has had occasion to associate the activities of a new meristem with the production of fatty acids.

When a healthy potato tuber is cut across and the cut surface left exposed to air, the cut surface is blocked within some 48 hours by a continuous deposit of suberin. The healthy living cells, immediately within the blocked surface entirely lose the dense masses of starch grains originally present in them. Throughout the tissue and parallel to the blocked surface a new meristem arises, cutting off new cells to the outside. These new cells thus left nearest to the blocked surface were originally normal meristematic cells, densely filled with protein, although apparently formed by utilization of a

carbohydrate reserve. They contain at first no visible fat, but they rapidly change in nature and micro-chemical examination reveals the intense production within them of fatty acids which are subsequently deposited upon their walls as the well-known suberin lamella. We have here a clear case of the disappearance of carbohydrate reserves being associated with intense meristematic activity giving new protoplasmic masses which contain, as by-products amongst their protein, fatty substances which are later released to the protoplast surfaces and then deposited upon the wall as oxidation and condensation products.

These considerations may be regarded at best as supplying simply a case for investigation, but it may be pointed out how open the problem is to attack. If the animal physiologist desires to study the fundamental fat metabolism of the growing cell he is opposed to great difficulties in isolating his living tissue under reasonable experimental conditions. The growing point of a seed or seedling is most readily isolated and permits of considerable experimental control, but so far as the writer is aware it has never been employed in studies of fat metabolism *in vitro*.

SUBSEQUENT UTILIZATION OF FATS LEFT BEHIND THE APICAL MERISTEM

Fats, notably the fatty acids, are utilized in various ways during tissue differentiation and it is probable that many of these processes are as yet unrecognized. It will suffice to point out very briefly certain of the possibilities that appear to be indicated by present experimental work: their elaboration in detail in some cases may be attempted in later papers.

(1) It has been already pointed out that in all cells the fats appear to migrate outwards and to find their way in large part on to the cell membrane. The exact extent to which they are held in the membrane depends upon various factors amongst which the most important external one appears to be the $\frac{Ca}{K.Na}$ ratio in the inorganic salts supplied to the plant. In soils poor in calcium the fats remain more mobile in the membrane and diffuse more freely to the surface, as indicated for instance in the thick cuticle and heavy suberin layers of the endodermis in the plants found growing upon peat (Priestley and Hinchliff(28)). Hansteen-Cranner(8, 9, 10) has some very valuable data as to the effect of K, Na and Mg in leaching out those fatty substances (as also the pectins) from the walls, whilst Ca on the other hand retains them firmly in the membrane.

(2) The phloem, because of its alkaline reaction, appears to free itself completely from fat within the membrane, though at various times fats will be detected in the contents. The freer diffusion of these fatty acids from the phloem is also indicated by the constancy with which Casparian strip and suberin lamella in the endodermis (Priestley and North (25)), both structures formed in part from fatty acids, form in position opposite the phloem in the root before they form opposite the alternately placed xylem groups.

In the xylem, the fats have also deposited in part in the wall, but the xylem during differentiation is notably acid in reaction and appears to lose mainly its basic constituents. When finally mature, the original cellulose membrane is strongly "lignified," a word that chemically is still of vague import. Lignified tissues are predominantly "methylated" as the phrase "wood spirit" for methyl alcohol itself implies. The writer would therefore tentatively raise the question to what extent the base choline, when lost to the cell contents, has left its methyl groups at least upon the membrane of the future xylem element.

(3) As the fatty acids migrate outwards from the differentiating tissue, they are carried with the movements of the sap along the wall of the surrounding tissues. The writer has traced elsewhere (27) the deposit of unsaturated acid upon the Casparian strip of the endodermis; they also appear in the exodermis of the root or the cuticle of the stem.

The exact position of these deposits, at the surface as cuticle or on interior walls as exodermis, may be connected with a number of factors. One appears to be internal and developmental, for it has been shown elsewhere that unsaturated acids are apparently picked up chemically by cell walls if these are at an early stage of development and in contact with oxygen. Another appears to be external, and the exact position and appearance of the exodermis has been shown in some experimental work, as yet unpublished, to be in part connected with the $\frac{Ca}{K.Na}$ ratio in the salts diffusing inward from the soil.

Wherever the fatty acids may finally deposit, in cuticle or cork layer, in exodermis or endodermis, their subsequent fate seems to be the same. The unsaturated acids undergo oxidation and condensation and the general result is the formation of a relatively waterproof layer, the fatty constituents of which have mainly lost their power of dissolving in fatty solvent and are changed in the same

general way as are the oils in the industrial processes such as the linoleum and varnish industries which depend upon the "drying" qualities of vegetable oils (Fahrion (6)). Incidentally it may be anticipated that the further study of the extraordinarily efficient methods by which these oils "dry" in their natural plant environment may prove to be not without interest to such industries.

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THE FACTORS GOVERNING BUD FORMATION: A CHAPTER OF PLANT PHYSIOLOGY

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CONTENTS

(To be continued)

INTRODUCTION

In current horticultural practice many methods of treating fruit trees are constantly being employed, the results of which depend upon the response of the buds to some manipulation of the shoot portion. Common operations of this kind are pruning, grafting, defoliation, and girdling or ringing. In every case the aim of the horticulturist is expressed as a desire to "produce more fruit buds"; and this aim is of so much importance economically that it has elevated the subject of the developmental physiology of the bud to a very prominent position in the programme of a horticultural experiment station.

Despite its importance it is only of late that it has become possible to contemplate a considerable increase in our knowledge of the growth relations of the bud. The explanation of this is that, until quite recently, investigation of the problems of the orchard has been confined to the plantation in the form of plot trials in which only rough quantitative methods have been possible, and in which the basis of judgment was usually the quantity and quality of the crop yielded by the various plots under treatment.

Although the method of the plot trial has been so developed as to make it suitable and accurate for certain subjects of research, opinion has gained ground that its inherent limitations render it an insufficient means of gaining an accurate conception of the relation, in normal response, of the perennial plant to a complex of external factors. To this must be attributed the fact that with respect to several important horticultural problems, *e.g.* the value of bud-selection, diametrically opposed views are held by leading workers in horticultural science.

When it is considered that these opposed views are always supported by a large body of data, painstakingly acquired from the results of plot trials, it is evident that something is lacking either in the method or the basis of judgment if not in both. And so the conclusion has now been reached by those engaged in horticultural research that field work in the plantation must be supplemented, or even replaced, by work in the laboratory where the methods of the plant physiologist and biochemist can be applied to smaller numbers of objects and where a more accurate knowledge can be gained of the factors which are in reality limiting the activity of the plant in response to a complex of controlled external factors.

Advances on the part of the plant physiologist to assist in elucidating the problems of the orchard have, until recently, been few in number and limited in scope. During the last decade, however, the research stations of the United States have combined, even if not intentionally, to remove this disability under which horticultural research has suffered and several problems have been attacked from the true physiological standpoint. In this country the fact that the position has been reached where long and careful investigations in the field have failed to give a rational explanation of the developmental conditions of fruit and leaf buds, or to complete an analysis of the factors which govern the formation of either, has stimulated advances in the same direction.

It is with the idea of presenting a broad and general survey of the progress made by the plant physiologist in this particular field that the following summary is offered.

CHAPTER I

THE SAP CONCENTRATION FACTOR

It has long been considered that the performance of a bud, during the earliest stages at least, is principally conditioned by the quantity and quality of the sap of the mother shoot and many attempts have been made to demonstrate the existence of a causal relation between the two. Barker and Lees (1916), at the Long Ashton Research Station, carried out extensive investigations with this object in view of the shoot growth and formation of buds in the apple, pear and plum. These were primarily economic in aim being occasioned by a desire to ascertain the degree of control which could be exercised upon fruit bud formation in view of the urgent need for such methods in the district where the research station is situated.

Here there is a striking difference between the type of shoot growth of fruit trees and that of other fruit growing districts. This is the tendency to form "bare unfurnished wood," *i.e.* for the lateral buds in the basal and mid regions of a growth-extension shoot either to remain dormant or, after making a small amount of growth, to die away altogether.

Different methods of pruning had already failed to modify this tendency for when apples were hard-pruned, *i.e.* when two-thirds of last year's wood were removed, the number of lateral buds to grow out was approximately the same as when but one-third was removed, as in lightly pruned examples. In both cases not more than six or seven good buds were left on a pruned last year's shoot. The net result was that practically the same number of leaf and fruit buds was given by both systems although, as would be expected, the light-pruned bushes formed much more of the bare wood.

This is in striking contrast to the results obtained by similar operations elsewhere and Barker and Lees came to the conclusion that, as an explanation was unlikely to be furnished by the results of any system of pruning, which could never have more than a local significance, an investigation of the ultimate causes of fruit bud formation was essential.

An extensive series of measurements of the growth-extension shoots of apples was therefore carried out and the meteorological data over a number of years examined. This led to the conclusion that the climatic conditions at Long Ashton, unlike those of other fruit-growing districts, remained favourable for continuous growth

until very late autumn when a deficiency of sunlight combined with a low temperature prevented proper ripening of the wood. They observed, in unpruned shoots, that the development of lateral buds varied inversely with the activity of the terminal, the latter in their opinion being the factor which decided how much "sap" should remain for the use of the lateral buds. They were careful to explain that such terms as "sap" and "sap-pressure" were merely employed as general descriptions of unanalysed complexes of physiological factors.

When the terminal bud was checked in growth during the season, either normally or artificially, it was found that fruit buds were formed behind the region so checked. On the other hand, active extension growth during the latter portion of the growing season took place at the expense of fruit bud formation.

The following argument was therefore developed to account for the peculiar behaviour of unpruned shoots at Long Ashton.

(1) Numerous growing-points, some active some dormant, each located in a bud, are distributed over a tree.

(2) Any of these buds may form a vegetative shoot or a fruit bud or remain dormant.

(3) The course of development of any is decided by a stimulus, absent or inactive in the case of dormant buds, which actuates cell-multiplication in the growing-point.

(4) This process being largely a matter of nutrition, the degree of activity of any growing-point depends upon the supply of "sap" reaching it.

(5) Under Long Ashton conditions only a small number of lateral buds receive this supply, resulting in strong extension growth of the terminal and failure of the lower lateral buds.

(6) Strong extension growth indicates excess of water, *i.e.* dilution of "sap" in the tree.

(7) A higher sap-concentration leads to a better balance between extension growth and fruit bud formation.

Since the publication of this argument the results of actual investigations of this sap-concentration factor have become available. Chandler (1914) carried out a series of sap studies on horticultural plants but, unfortunately, the methods of extraction employed were somewhat primitive and the process so lengthy that it is difficult to estimate the precise significance of most of his data. His first important conclusion was that, generally, more than half the osmotic pressure of the sap, both of the cortex and leaves, was due to sub-

stances other than sugars and electrolytes. In order to arrive at this conclusion the total molar concentration was determined by means of the Beckmann apparatus. The osmotic pressure due to electrolytes was then calculated from the strength of a solution of potassium chloride having the same conductivity as the sap. Separate determinations were made of the total sugars and, after calculating the molar strength, the proportion of the osmotic pressure not due to electrolytes could be determined.

Apparently seasonal variations occurred but the data given are insufficient to do more than support the general conclusion that, during the season of rapid growth, the sap-concentration of the cortex was lower than at other times. This was connected, in the case of the asparagus, with an increased moisture content but similar observations were not made for any of the fruit trees worked with.

Partly developed leaves of apple and peach were found to have a higher sap-concentration than the young leaves near the growing-point; a state of things found previously to exist in the leaves of Syringa and other shrubs by Dixon and Atkins (1915).

Chandler showed that the molar concentration of the sap was a fair measure of the nutrient condition of the plant tissue. A much weaker concentration was found in the roots of ringed trees than in those of similar unringed trees. He also showed that defoliated trees contained in all parts a smaller supply of material in solution than similar trees the leaves of which had been allowed to remain.

There is therefore in Chandler's work a certain amount of evidence for the existence of the negative correlation between sap-concentration and quantity of extension-growth suspected by Barker and Lees. Actually it has been demonstrated in the case of young trees of walnut and apricot by Reed (1921) whose methods are much less open to criticism. He expressed the sap from entire shoots after these had been kept in a freezing-mixture for 18 to 24 hours. The osmotic pressure of each sample of sap was then determined from the lowering of the freezing-point, while, concurrently, the growth increment of the trees was measured at seven-day intervals on other selected shoots.

In the walnut a not altogether satisfactory correlation of $(-0{\cdot}557 \pm 0{\cdot}113)$ was established between sap-concentration and growth increment, but a stronger correlation was found in the case of the apricot where $R = (-0{\cdot}613 \pm 0{\cdot}079)$. Reed claimed therefore that the connection between rapid growth and a lower sap-concentration was thereby established.

The concentration of the sap of rapidly growing shoots of a heavily pruned tree was lower than that of the slower growing shoots of an unpruned tree and this was attributed by Reed to the diminished demand made by the latter on the plastic materials of the tree permitting a greater accumulation of soluble materials in their tissues. The higher concentration was also contributed to by the smaller water content of these shoots. In both classes of shoot the concentration was found to increase with the advance of the season, an increase referred by Reed to the photosynthetic activity of the leaves. The water-content of the soil was also found to influence the concentration which fell after the application of irrigation water.

Contrary to the general belief, Reed showed that the sap in the apical portion of the shoot was more concentrated than that of the basal region. This led him, in a discussion of the relation between concentration and fruitfulness, to develop the thesis that, as the buds for the following year are developing during the period of slower growth and higher concentration, the connection between such concentrations and fruit bud formation could not be doubted. To account for the paradoxical state of things in which, at one and the same time, the concentration of the whole shoot is lower during periods of rapid growth but yet that of the rapidly growing apical portion is uniformly higher than that of the slowly growing basal region, in spite of the fact that the higher water-content of the former necessitates a correspondingly higher content in solutes in order that this relation may be maintained, he found it necessary to introduce a qualification. He advanced the opinion that growth is regulated by the quality as well as the concentration of the sap, and that a qualitative distinction is supplied by the absence from the apical region of growth inhibiting substances. More rapid growth in this region was thus accounted for. He regarded the presence of such substances as proved by Loeb for *Bryophyllum*, and by himself for the pear, but still retained the conviction that the vegetative growth of a shoot was retarded by an increased sap-concentration and *vice versa*.

The possibility of a certain degree of control of the sap-concentration was adumbrated by Wiggans (1919) who ascribed to it great importance in the resistance of buds to the effects of frost and also for the production of fruit buds and blossoms. He considered that a maximum sap-concentration with an accompanying degree of frost-resistance could be maintained by appropriate pruning methods and soil-management. He showed that the sap from bearing

spurs had a slightly higher concentration during a considerable portion of the year than sap from non-bearing spurs, both sugars and starch being present in larger quantities in the former. On removal of the blossoms from a bearing spur the available sugar was apparently directed towards the development of a fruit bud for the following year.

Before the work of Chandler and Reed was accessible Barker and Lees had already come to the conclusion that the sap-concentration factor was inadequate to account for all the abnormal phenomena encountered in connection with bud growth. The work of the two first named actually does little more than confirm this conclusion, for, while it may be granted that their results establish generally the negative relation dealt with above, this is but a short step towards the elucidation of the problem of bud formation and growth. The biochemical foundation of the relation still remains undetermined, while its causality has still to be established. Furthermore, the physiological consequences of such a relation are far from obvious as no doubt Reed felt when, in order to account for the basipetal concentration gradient in the sap of a growing shoot, he fell back on an undetermined qualitative factor or inhibitor.

It is impossible to foresee such a relation as the above being established before our knowledge of the local distribution of the sap solutes is greatly increased. Curtis (1920) certainly claims to have obtained evidence that one of several factors concerned with polarity and inhibition is associated with such a local distribution, but up to the present this has not been published. Apart from evidence of this kind it is essential to know whether all the lateral buds are of equivalent potentiality at the beginning of the season and of the same periodicity during it. At the beginning there are obvious size differences to which it is difficult to attribute any biological significance and, although it is generally assumed that the smaller basal buds could in the absence of the inhibiting influence make an equivalent amount of progress to that made normally by the larger buds at the distal end, close observation leads to the conclusion that this is not necessarily the case. Pruning experiments performed by the present writer in 1922 involved the cutting back hard of last year's shoots to about five or six visible buds, including the most minute dormant buds. In every case at least two of these could not be forced out by pruning, while the next one above formed a tiny spur with four or five reduced leaves. On the other hand, the next two above appeared capable of giving rise to shoots as large as those furnished by the

distal buds of unpruned shoots: this was especially the case with the plum. When forced into growth the basal buds generally furnish weak spindly shoots the character of which can be recognised at a distance several years afterwards.

It is perhaps unnecessary to mention that studies of the sap-concentration have so far shed no light upon the nature of the morphogenetic factors responsible for the differentiation of fruit and leaf buds respectively; nor has the quantitative study been brought to a stage where it is possible to state the relation between the growth of the lateral buds and the quantity of sap furnished to each. Farmer (1919) has shown that, in the ash, both the absolute and specific conductivity of the wood for water fall off rapidly as the apical region is approached. To this phenomenon he connected the characteristic growth habit of young ash trees in which the apex often dies back for a considerable distance, the elongation being taken up by one of the stronger laterals. The case is different with other trees, *e.g.* the sycamore, in which the conductivity figures correspond with the persistence of apical growth.

Having reached the conclusion mentioned above, Barker and Lees (1919) carried out a detailed investigation of the processes involved in the normal growth of shoots of the apple and pear. They considered that these could be pictured more easily if the tree were regarded as a "colony of competing growing points" rather than as a single organism. Other workers, like Harvey (1921), have held that this idea of individuality of growing points may be pressed too far, the probable truth being intermediate between this and that of the individuality of the spur. For the time being, however, the former is more helpful and possesses the advantage of simplicity.

Continuing observations upon the buds on the last year's shoots of pear they distinguished three stages of growth during the season.

(1) All the buds which are not dormant break and show uniformly a short length of young leaf. The stage is independent of root-action and is conditioned by a preliminary rest period followed by a rise of temperature.

(2) The terminal bud gains precedence and the apical buds grow faster than those near the base. At the end of this stage about four of the apical buds are in active growth but the basal ones have stopped.

(3) Most of the buds have ceased growth but a few of the apical buds are elongating to form woody shoots; the terminal is the last to stop.

Being unable to account for this behaviour on physiological grounds they thought that the recently published growth inhibition hypothesis of J. Loeb might possibly contribute towards an explanation and an extensive digression is necessary at this point in order to give a summary of Loeb's works previous to a consideration of the extent to which they assist in explaining the difficulties encountered by Barker and Lees.

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CHAPTER II

THE WORK OF J. LOEB ON *BRYOPHYLLUM CALYCINUM*

Loeb's latest-developed and most widely-known theory of inhibition was finally developed as a more satisfactory explanation of most of the foregoing phenomena of bud growth and dormancy than any based on the presence or transport of nutrient materials. According to this theory the growing apex of a stem forms a definite substance (inhibitor), antagonistic to bud growth, which, migrating towards the base, impedes or inhibits the growth of the lower buds into shoots. The apical buds are the first to be freed from this and begin to grow out, at the same time producing fresh supplies of inhibitor the concentration of which increases gradually towards the base. The subapical buds are therefore retarded in growth increasingly in this direction until, finally, the point is reached where the concentration is great enough to inhibit lateral shoot-growth completely.

Loeb (1915–1918) chose as the object of experiment the tropical succulent *Bryophyllum calycinum* which, in addition to axillary buds,

produces buds in the notches of its leaves. This plant was considered to be especially favourable for an investigation of correlations of growth and inhibition phenomena and Loeb claimed as the outcome of his work the determination of a number of rules governing these classes of phenomena which were so simple that they formed "a securer basis for hypothesis than is offered by most former experiments in this direction which have not led to such simple rules."

Normally, if a leaf is detached from a plant of *Bryophyllum* and laid upon wet sand it quickly puts out roots and shoots from its notches. While the leaf forms part of a healthy plant this does not usually take place nor do the axillary buds grow out to form shoots. If, however, the flow of substances in the plant is abnormal, as when either the roots or shoots have suffered injury, a growth of shoots may take place in moist air from the notches of leaves which are still in connection with the plant. It should be remarked that the accuracy of these premises (which are those of Loeb) has been disputed by Miss E. L. Braun (1918) who claimed to have observed frequent instances of shoot formation from the notches of the leaves while these were still in connection with the plant. Accompanying her note on the subject is a photograph of one plant showing abundant shoot growth in such a leaf, the plant having received no special treatment. She further stated that in isolated leaves the number of notches to produce leaves in moist air may vary from one or two to all. In his reply Loeb (1918 (i)) did not deal with the latter point but asserted that this plant was a sick and therefore abnormal specimen. The photograph certainly shows it to have been of more than usually straggling habit and its stem appears abnormally weak. Loeb considered that the bend in this weak stem acted as a partial block causing the upper part of the stem to behave as an isolated portion the leaves of which are destined to give rise to shoots.

There appears to be no good reason for holding that the portion of a stem above a simple bend is isolated physiologically, nor do many of Loeb's own experiments on growth curvatures bear this out. Moreover, Goebel (1908) has shown that if all the shoot buds are removed the buds on the leaves will grow out. He explained this by saying that, as the stronger points of attraction are no longer present, the "building material" is drawn towards the points of weaker attraction, *i.e.* the growing points of the leaves.

It is impossible to give in this place more than the briefest summary of typical portions of Loeb's work and details must be sought

in his numerous papers. It will be convenient to study the pertinent portions under the two headings below.

(I) The Mechanism of Growth and of its Inhibition (1915, 1916 and 1917 (i)).

In the first series of experiments use was made of isolated leaves or of single nodes with one or both axillary leaves intact.

At the outset Loeb definitely excluded the idea that the wound stimulus might be responsible for the regeneration of buds because of the fact that, in *Bryophyllum*, this is not localised at the wound although the conditions there are responsible for the healing of the latter. There is not a great deal of force in this objection, for it has been shown repeatedly that regeneration is not necessarily so localised. Jost (*Plant Physiology*, English edn. p. 39) pointed out that, in isolated Begonia leaves, buds may arise from uninjured epidermal cells situated at some distance from the wound callus as well as from the epidermal cells of the callus itself.

If definitely formed growing-points are already present in the vicinity of a wound, regeneration is most likely to take place earliest there and it is well known that a bud may be stimulated to growth by an appropriate wound made near to it. Thus Jesenko (1912) was able to force into growth the buds of previously defoliated trees of *Tilia* and *Fagus* by puncturing the bases of the buds and also by injection into the bases of alcohol and ether in suitable strengths. Further, Ross (1912) showed that in the leaf of *Conostegia subhirsuta* the adventitious leaflets which arise on the upper epidermal surface, following a presumably chemical stimulus due to infection by eel-worm, may develop at some distance from the point of attack as though in response to a conduction of a growth stimulus. Ross was unable, certainly, to reproduce these structures by ordinary wound stimuli but, in the present state of our knowledge, it is hardly possible to classify wound stimuli as transferable and non-transferable respectively.

Loeb employed single nodes which were either suspended in moist air over water or placed with one or both leaves actually dipping into the water. In the latter case buds developed in the submerged apical notches only, while the axillary buds remained dormant. He pointed out, however, that, while holding for this set of experiments which was carried out during winter in a greenhouse, this is not always true, for these buds often developed normally in the spring.

When suspended over water no buds grew out if the leaves were small, but if these were large buds developed in some of the notches

while one of the axillary buds also grew out. In another experiment he removed one leaf leaving the other intact and found that the bud in the axil of the detached leaf grew out and also—sometimes—a few of the dormant buds in the notches of the remaining leaf. No roots were formed at the node itself.

Turning next to the isolated leaf Loeb found that, when suspended with its apex dipping into water, the submerged buds at the apex sprouted first. If suspended in moist air, however, those nearest the thick fleshy middle portion developed first. If the isolated leaf were cut into as many portions as notches, so that each portion contained one notch, and these were laid on water then every bud developed.

In a set of experiments where one leaf was removed from a node and, in addition, the upper or both axillary buds were removed, the following results were obtained when the apex of the leaf dipped into water.

(1) Both axillary buds removed: 12 leaves out of 18 formed adventitious roots or shoots.

(2) Upper axillary bud removed: 4 leaves out of 10 formed adventitious roots or shoots.

(3) Both axillary buds remaining: No leaf out of ten examples formed either roots or shoots, but in every case the upper bud grew out into a shoot.

(4) Isolated leaves: all formed adventitious roots or shoots.

From all these results Loeb concluded that, even after removal of the buds, the stem still exercises an inhibiting influence upon the formation of roots and shoots in the notches of the leaf and that, if the buds are not removed, the growth of the one opposite the leaf enhances the inhibiting effect considerably.

In order to confirm the above conclusions Loeb performed similar experiments in which he employed, instead of isolated leaves, pieces of stem each containing two or three leaves and having one pair of leaves remaining. These were partly submerged in water with the following results.

(1) Basal pair of leaves left: new shoots formed from the apical buds within a few days.

(2) Apical pair of leaves left: either no shoots at all were formed from the stem buds or this took place after considerable delay.

Bryophyllum had previously often been investigated by experimental morphologists and Loeb investigated the conclusions of De Vries (1891) and Goebel (1908) who both concluded that the growth of roots and shoots in the leaf was inhibited by root-formation in

the stem. The two views varied slightly for while Goebel thought that the root-formation itself was the inhibitor, De Vries considered that this was to be sought in the root-pressure or flow of water caused by it.

Loeb inclined towards the latter view as the result of a series of experiments upon detached leaves each with a fragment of stem attached. In moist air not only did this fragment form roots but the leaf also formed roots and shoots. As there was no root-pressure in this case he concluded that the view of De Vries was supported by this result. In water the result was different, for the stem formed numerous roots while the leaf had few or none, showing, according to Loeb, that roots formed on the stem have an inhibiting effect upon the leaf if they can produce root-pressure.

When an isolated leaf was hung sideways in a vertical plane it behaved differently according to whether it was suspended in moist air or in water. In the former case shoots and roots developed mainly from the lower margin near which there was an accumulation of red pigment. In the second case roots developed from the upper margins as well.

In a discussion of the conditions in which the growth of the axillary buds is inhibited or accelerated as the case may be, Loeb adduced evidence that the leaf favours the growth of the opposite axillary bud whilst inhibiting the growth of its own. This idea has, however, to be restricted because, in order that this growth may take place, a water supply has to be furnished to enable the bud of an upper node to grow out while this is unnecessary in the case of those of a basal node.

The outcome of all these results was the provisional assumption by Loeb that "the growth of the bud depends upon the flow of certain substances from the leaf to the bud. That bud which receives these substances first will grow out first and thereby prevent the flow to the other buds whose growth is thereby inhibited. The apparent inhibition of growth in one place is simply due to the fact that, under the conditions of the experiment, the substances required for growth flow to some other place and are retained there. The removal of this inhibition consists in creating conditions which force the substances to flow to the places where we want growth to occur."

Loeb next turned to the question of the rules governing the mechanism of this process of inhibition (1918 (1) and (2)). He showed that if the base and tip of a main stem were amputated and the leaves of the remaining nodes removed, only the buds at the highest apical

node would grow out when the shoot was suspended, either upright or inverted, in moist air. The buds at the base were all in this way inhibited for, if isolated, they were all capable of growing out.

The greater the number of nodes left below the apex the more quickly did regeneration take place and consequently Loeb supplemented his first provisional assumption by asserting the validity of the following relation governing the process. "If an element '*a*' inhibits the growth in an element '*b*,' then '*b*' often accelerates or makes possible growth in '*a*.'"

Confirmatory evidence was held to be furnished by experiments upon isolated nodes suspended in moist air which showed that even a small portion of leaf makes possible the growth of the opposite bud, the leaf of which is removed, just as the growth of this shoot inhibits the growth in the notches of the latter when this dips into water.

In another experiment a piece of stem was stripped of all its leaves and suspended in moist air. If it consisted of several nodes the two most apical buds grew out, but growth was greatly accelerated if the basal leaves were left on. This led Loeb to regard the inhibition as consisting of a receipt from the inhibited part by the dominant of a "something" which accelerates growth or renders it possible. An isolated node from near the apex with leaves removed cannot, according to Loeb, regenerate in moist air, but can do so if laid upon water, showing that water may be the "something"; but he held that the water was only indirectly necessary to render possible the flow of material. To account for the fact that a similar node isolated from near the base is able to grow out in moist air he advanced the explanation that such a piece does not dry out so easily as one from near the apex.

At this point, in discussing the nature of the mechanism of inhibition, Loeb assumed for the comprehension of the above rules, the existence of a flow of certain (possibly specific) substances (or formed cells) from the places where the dormant buds are ready to grow out, or the prevention of such a flow to these buds. In support he quoted the results of experiments in which the notches on one side of a leaf were isolated from the leaf and from one another and then placed in water. All the isolated notches grew out into shoots while only three of the notches left on the leaf grew out. If the isolated portion were small, growth might be feeble or nil, while the rate of growth was greater in a shoot from a whole leaf than in one growing from an isolated notch.

Loeb asserted that these observations became intelligible only on the assumption that the leaf furnishes a flow of certain substances which, reaching certain notches, cause shoot and root growth. Growth would also occur if these substances were already present near a bud and were prevented from flowing away, while, conversely, the growth of new shoots might deviate this flow as in the case of an isolated piece of stem deprived of leaves and laid upon moist soil. In this case shoots grew from the top buds and, shortly afterwards, the piece of stem above the top node began to wilt up to a point a few millimetres from the node. This shrinkage did not take place if the root of the stem was left intact, the flow of liquid being maintained by the root pressure.

Finally, Loeb mentioned the great influence that light exercised upon the growth of the notches of an isolated leaf. Suspended in moist air none of the notches grew out in the dark but proceeded to do so promptly when exposed to light, provided the sojourn in darkness had not been too prolonged. With leaves submerged in water the growth of the notches was very much less than in the dark.

Discussing the whole question of growth and regeneration on the assumption that a leaf notch can only grow out if there is no flow of shoot-forming material away from the notches, Loeb maintained that in the living plant, where the circulation is normal, these conditions are not present. (He relied partly upon his demonstration that root-pressure is a factor influencing this flow and adduced supporting evidence from his first experiments with isolated leaves where the flow away from the notches was prevented.) As a consequence, one or more of the notches growing out exercise a "suction" effect on the flow of liquids in the leaf and inhibit the growth in other notches, especially if the opposite bud can grow out and increase the suction effect.

He also considered that this idea of a deflection from the leaf to the opposite side of the stem is borne out by the following facts:

(1) The bud opposite a leaf grows out rapidly if its own leaf is removed, while the growth of the bud whose leaf remains is inhibited.

(2) The deflection ceases when the stem is split, and the leaf ceases to inhibit its own axillary bud.

(3) The leaf attached to a split node, when immersed in water, produces shoots rapidly from the notches.

(4) In a node with both leaves attached the flow is deflected from both axillary buds and neither grows out.

The failure of the notches to grow out while the leaf is in connection with the plant Loeb ascribes to the flow of material from the root and leaves into the stem and on to the apical end carrying material away from the notches which are thereby prevented from growing out. Thus, it is not the isolation itself which causes buds to grow on a part where otherwise they would have remained dormant while in connection with the plant, but the fact that material can flow to and be retained in parts where formerly this was impossible.

In reaching the above conclusions Loeb appears to have been influenced by his previous work on the regeneration of *Tubularia* where he demonstrated that the formation of the aboral polyp was delayed by the growth of the oral polyp. But in *Tubularia* he showed definitely the existence of special cells which, carried in the general circulation from the entoderm, collected at the spot where regeneration was about to start. In *Bryophyllum*, however, the presence of an analogous flow of substance has yet to be demonstrated.

This section may be concluded by setting out Loeb's primary hypothesis of bud development and dormancy as follows:

(1) The question of a bud's dormancy or development is decided by the amount of certain substances at its disposal. Under normal conditions these flow to the growing-points and only when a portion is isolated is this flow prevented and some buds obtain sufficient of the substances to begin to develop. Those that develop first set up a "suction" and monopolise the supply inhibiting the growth of the others.

(2) The photosynthetic activity of the leaves may play an important part in the regulation of new shoot formation.

(II) Phenomena of Geotropism and Polarity (1917 (iii) and (iv), 1918 (i) and 1919 (i) and (ii)).

In this second phase Loeb studied the above phenomena in severed segments of stem having four to eight nodes. These were suspended horizontally in moist air and gave the following results.

(*a*) All leaves removed: There was feeble development of shoots at the apex and of roots at the base. The geotropic curvature was slight and distributed generally along the length of the stem.

(*b*) A single leaf left attached to the apical node: Shoot formation was still retarded but root production and geotropic curvature were enhanced.

(*c*) A single leaf left attached to the basal node: Shoot formation was accelerated at the apex but root production and geotropic curvature were reduced and restricted to the basal region.

In all three roots were produced mainly on the under side except at the base.

The following hypothesis was developed to account for these results. Each leaf has a tendency to send specific shoot-forming substances towards the apex and root-forming substances towards the base. Associated is a hypothetical geotropic hormone. Both root-forming substances and hormone are sent by the apical leaf towards the base and tend to collect on the lower side, while the basal leaf exerts a "suction effect" upon them. In *Bryophyllum* the hormone is most likely associated with, or even identical with, the root-forming substances but this is not necessarily the same with all plants. In some it might possibly be associated with the shoot-forming substances.

In all these experiments on geotropic curvature no account was taken of purely mechanical considerations. Loeb's stem-portions which were suspended horizontally from vertical threads attached to the ends were, in effect, beams suspended freely from two points. They possessed a property additional to those of a physical beam in the capacity of the neutral axis to elongate owing to the growth in length of the stem-portion during the experiment. This forced the suspensions out of the vertical in time thereby introducing a horizontal component of their tensions, which, together with the bending moment of the stem portion, would account for much of the curvature exhibited by the latter. This is borne out by the few figures for growth-extension given by Loeb and also by the photographs shown (1917 (iv)).

It is not without interest to follow the sequence of ideas which culminated in the adoption of the Inhibition Hypothesis by Loeb. Originally he regarded the inhibition of the growth of a bud as due to a kind of unfair competition on the part of some other growing-point, which, growing out first, exerted a "suction" upon certain hypothetical growth-producing substances flowing from the leaves to the buds.

This concept was subsequently modified and the inhibited portion regarded as the centre of production of another hypothetical "something" which, received by a dominant portion, either accelerated growth or rendered it possible. The flow of this "something" might conceivably be deviated by new and vigorous shoots arising from other growing-points.

After his study of geotropic curvature Loeb divided his growth-promoting substances into two distinct categories as specific shoot-

and root-forming substances respectively. The hypothetical geotropic hormone was considered to be generally associated with the latter.

The sequence was concluded by the elaboration of the Inhibition Hypothesis and, in this, growth-promoting substances were left entirely out of consideration, a single active substance being assumed as the inhibitor.

Experimental evidence has been obtained by other workers apparently supporting the hypothesis on the one hand and on the other definitely controverting it. Before considering this in detail, or the validity of the evidence from Loeb's own experiments, a return will be made to the work of Barker and Lees, certain aspects of which they considered were satisfactorily explained on the assumption of the action of an inhibitor such as that of Loeb. This action they called the "Loeb effect."

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CHAPTER III

THE LOEB EFFECT IN FRUIT TREES

Having considered the Loeb hypothesis, Barker and Lees (1919) inclined to the opinion that the production of an inhibitory substance by the terminal bud during the second growth-stage, when growth conditions were steadily becoming more favourable, might account for the bringing to a standstill of most of the lateral buds. They thought that in the first stage, when growth conditions were less favourable, the "Loeb effect," *i.e.* the effect of the inhibitory substance produced by the terminal bud, could not be very considerable. They were not fully convinced, however, that this "Loeb effect" was the sole factor in the case of the pear twigs under observation, for, if such were the case, the shoots at the end of the year should have shown a few wood shoots near the apex, followed by a series of wood spurs becoming progressively weaker towards the base of the shoot.

In the actual case they found a graduation far from perfect, extra strong spurs or shoots occurring out of position. They postulated, therefore, the operation of a "bud factor," in addition to the "Loeb effect," and adduced the following evidence for its existence.

(1) In winter the three or four buds at the base of the shoot will be found to be smaller than the rest, and if size is any index of strength they are weaker than normal.

(2) By forcing water through the conducting tissues of a shoot, after breaking off a bud, large flows were found in the case of a bud near the apex—the sub-terminal excepted—and very small flows in the case of the basal buds.

(3) In certain ringing and notching experiments a bud forced into growth made progress in proportion to its original strength and not to its position on the shoot.

(4) In apple and pear shoots kept in water in a warm place one or two basal buds near water-level made appreciable growth in spite of the "Loeb effect" whilst a number of buds between these and the apex remained dormant.

They arrived as a result at the following explanation of the various stages of growth.

Stage 1. The quantity of growth made by each bud is largely a question of its inherent strength which may possibly be governed by its vascular supply. The initial stimulus to activity is a rise in temperature.

Stage 2. The conditions of growth become more favourable, the "Loeb effect" begins to tell and the lower laterals are arrested unless they are strong.

Stage 3. Normally growth takes place only at a few apical buds. In abnormal seasons laterals which have been arrested may recommence growth owing to a lessening of the "Loeb effect" if the growth at the apex is checked or to an increase of "bud factor" due to increase of "crude sap."

They discussed, in addition, what they called the "variety factor." Not only was there a difference, so far as bud-break was concerned, between apples and pears as a class but within these classes there was every transition from the variety which broke well, *i.e.* had few dormant buds, to the variety which broke badly.

Finally they summed the factors influencing the growth of buds on a last year's shoot as the following five and it is of interest to note the stage of analysis reached which is indicated by the fact that only one is measurable: they are (i) temperature, (ii) Loeb effect, (iii) bud strength, (iv) root action and (v) variety factor.

Pursuing a different line of investigation Barker and Lees (1919 (ii)) investigated the effects of notching and ringing upon bud formation and development. It was assumed that an inhibitor, flowing from the terminal region backward by way of the cortex, might be prevented from reaching certain buds. There would thus be no obstacle to the development and further progress of these.

Their original scheme was to experiment with rings by which the tissues external to the cambium were removed in widths varying from $\frac{1}{16}$ inch to $\frac{1}{4}$ inch. Knife-edge rings, by which no tissues were removed, were also employed. It was decided to cut rings of each kind

(*a*) upon the main stem, main and subsidiary branches and on last year's twigs;

(*b*) in spring, summer, autumn and winter;

but the late season ringing had to be abandoned owing to the danger of infection of the wound by canker. The results of the May or spring ringing were as follows:

(1) One or more shoots appeared below the ring, the greater the width of the ring the larger the number of shoots. The new shoots were long and spindly.

(2) Callus soon started forming over the wound from the cut edges, almost wholly from the upper edge of the ring downwards, and when the ring was completely covered growth in length of the shoots below the ring ceased.

(3) In controls, where the ring was waxed over, callus grew much more quickly, growth of the shoots below the ring ceasing when the callus had completely united across the ring.

(4) Knife-edge rings failed to cause dormant buds to break as also did interdigitating cuts which overlapped but were not made completely round the shoot.

The following results were obtained with last year's shoots which had been ringed in the manner indicated below.

(1) Rings in every internode: Buds grew out into spurs or shoots. On the whole the strongest came from the most basal buds but otherwise the strength of the shoot appeared to depend upon the initial strength of the bud.

(2) Rings in alternate internodes: The shoot immediately below a ring was stronger than that above, exceptions being due to differences in initial strength.

(3) Rings in every third internode: All the buds broke well but the third one below the ring was usually weak.

(4) Knife-edge rings: One or two buds immediately below the ring started into more active growth than would have been the case had no ring been made.

(5) Generally: Varieties that generally break badly showed but little effect after ringing, the variety factor being a real one. A pruned shoot ringed in every internode showed stronger growth than an unpruned.

In their notching experiments a crescentic slip of bark and phloem was removed from immediately above or below a bud with the following results:

(1) Notching above a bud: The bud affected made more growth than similarly situated buds on untreated shoots.

(2) Notching below a bud: Growth was prevented and the bud remained dormant.

All these results were what was expected on the assumption that a ring or notch interposes a barrier to a "Loeb effect" travelling downwards.

In their latest work Barker and Lees (1920) have considered the bearing of Loeb's hypothesis upon the results obtained by pruning according to the Lorette System. The work in question is highly technical in character and lends itself neither to summary nor quotation so that it cannot be dealt with here; but it may be said that the authors claim that the hypothesis not only explains Lorette's results in France, and the failure to obtain these by the same methods

in the wetter climate of Long Ashton, but also serves as a guide in the elaboration of a technique modified to suit these conditions.

We see therefore that Barker and Lees, being unable to account for many of the phenomena encountered during extensive observations of the growth of young shoots of fruit trees, accepted Loeb's Inhibition Hypothesis as a partial explanation of these. No critical test of the hypothesis was made; it merely appeared to fit the cases more closely than any explanation based upon current physiological conception of the processes of growth and nutrition. It was not accepted as a complete explanation and other factors were suggested as playing, in all probability, some part in the processes which give rise to the phenomena in question.

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CHAPTER IV

EVIDENCE IN FAVOUR OF THE LOEB INHIBITION HYPOTHESIS

The work of Loeb has stimulated a number of attempts to verify his conclusions with plants other than *Bryophyllum*. In one of these Reed and Halma (1919 (i)) suspended cuttings of the Chinese Lemon in moist air and observed that shoots developed from the buds at the apex and roots from those at the base whether the shoots were suspended normally or inverted. If the apical buds were killed those immediately below assumed their characteristic mode of growth.

When the shoot produced by the apical bud was removed a new one was formed by an accessory bud, not by a lower one. When the apical bud was enclosed in plaster of Paris the lateral buds grew out; otherwise these developed only when notched above, similar unnotched buds remaining dormant. This mode of development of the laterals was considered by Reed and Halma to support the theory of the presence of an inhibitory factor in the apical bud.

When the cuttings were suspended horizontally new shoots developed on the upper side alone. These were not confined to the apical region, *i.e.* there was no dominance of the apical bud.

Working with pear shoots the same two authors (1919 (i)) claimed that the fact that the young vertical shoots remained unbranched, owing to the inability of the laterals to grow out unless the apex were amputated, supported the hypothesis of a growth inhibiting substance travelling from the apical bud towards the base to produce dormancy there. They also demonstrated that shoots suspended horizontally behaved similarly to those of the Chinese Lemon and held that, in both cases, the dorsal buds were removed from the restraining influence of the inhibitor which, accumulating on the ventral side, produced dormancy there. This is one of the few cases on record where an attempt has been made to exercise a small measure of control over the action of the "inhibitor" in the presence of the terminal bud.

Recently Reed (1921) has returned to the subject in an attempt to analyse the growth phenomena and relationships of the lateral shoots of the branches of young pear trees. He claimed that the definite and orderly fashion in which new shoots were developed suggested that their growth and distribution were controlled by definite causal factors. He observed that the lateral shoots formed a series descending basipetally in order of magnitude. This rate of descent was rapid for the first half-dozen or so laterals but more gradual below these. Variability was found to be least in the distal laterals and to increase gradually to the ninth from the apex. Explanations of this characteristic mode of growth based upon the transport of food materials or ideas of "polarity" were considered unsatisfactory by Reed who maintained, further, that Child's idea of a physiological gradient (1920), while closely picturing the phenomenon, did not account for the production of the gradient. He considered that if it were a question of the distribution of nutrient materials in the parent branch it was difficult to understand why the growing points at the distal extremity of the branch should acquire such quantities as to result in the production of larger shoots, while the growing-points near the proximal extremity usually failed altogether to develop during the first season.

Reed considered that it was more logical to assume that the observed type of development depended upon the distribution of some substance antagonistic to growth which, after being formed in the distal region of the shoot, migrated towards the base.

According to this view, amputation of a portion of the mother shoot removes the most active centres in which this substance is produced and the buds immediately below the point of amputation

grow out first because they are the first to be freed from the inhibiting substance. As soon as the new shoots begin to grow they in turn begin to produce more of the inhibitor which tends to keep the lower buds dormant. The successively smaller sizes of the lower shoots can also be attributed to the inhibitor, each shoot in turn contributing to the effect with the result that the lowest buds of all are completely prevented from growing out.

This of course is merely a paraphrase of Loeb's theory of inhibition.

Reed considered that his data failed to support the hypothesis of a migration of growth-stimulating substances from the surrounding tissues to accumulate in the dominant area. The first laterals from shorter mother shoots made more growth than those from longer mother shoots yet the latter had a larger surrounding area to draw upon. He thought a case could be made out for the occurrence, in all the buds of a mother shoot, of growth-stimulating substances of a catalytic nature.

It will be remarked that Reed and Halma (1919 (i)) introduced an additional quality of the inhibitor, but it is not quite clear as to whether they intended this as a definite capacity for positive directive movement to the stimulus of gravity or whether they attributed to it a real ponderability. They confined themselves to describing how, under its influence, the substance collected on the ventral side of a horizontal shoot to inhibit the growth of the buds situated there.

Even if the palpable nature of the inhibitor and the reality of its mode of production in the apical region be granted, there are many difficulties in the way of accepting the special mode of transportation in the diffusion stream of the cortical region postulated by Reed and Halma.

Another research of the same character was carried out by Appleman (1918) upon potato tubers. He claimed to have shown that these contain a special growth-promoting substance and that a disease known as "spindling sprout," which is characterised by the production of long slender sprouts, may be due to an abnormally low content of this substance in the tubers. He asserted that normal growth was dependent upon the growing bud being surrounded by a mass of tissue containing sufficient of this substance and that his experiments showed the basal buds to be incapable of growing out until their connection with the terminal bud was severed. It is not clear as to how Appleman regarded the nature of this hypothetical substance except that he definitely rejected the idea of a chemical basis for it.

The present writer carried out similar experiments using tubers of Sharpe's Express, an early variety, and Scottish Farmer, a main crop variety. While the latter behaved in the manner described by Appleman, the contrary was the case with Sharpe's Express; growth of the basal buds of these occurred together with strong growth of the terminal.

The net result of the above researches therefore is merely a confirmation, with other material, of some of the results obtained by Loeb with *Bryophyllum*, but before testing the evidence from this the results of other workers which appear to be opposed to the Inhibition Theory will be considered.

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CHAPTER V

EVIDENCE AGAINST THE INHIBITION HYPOTHESIS

Evidence against the validity of Loeb's hypothesis is furnished not only by experiments definitely designed to test it but also by others which had not this object in view. For example, the possibility of a stimulus of a chemical nature being necessary to initiate the outgrowth of the buds of *Bryophyllum* is indicated by the experiments of Erwin F. Smith (1916).

With *Bacterium tumefaciens*, the organism of the crown gall, Smith made inoculations of this in the leaf axils of young plants of *Nicotiana*, *Citrus* and various other species. Tumours were produced as a result which were covered all over with diminutive, distorted and abortive leaf- or flower-shoots. In the case of the tobacco plant, inoculations were made in places where no bud "anlage" were known to exist, viz. in the midribs of leaves, but here also tumours arose bearing leafy shoots, the largest proportion being yielded by young leaves and none at all by those that were well developed at the time of inoculation.

Smith maintained that the changed stimulus had produced a

more embryonic and primitive condition in the rapidly developing young tissues, the stimulus in this case being pathological, in contrast to the normal physiological growth stimulus which becomes active, for example, when the top of the plant is removed and produces the normal physiological response of regeneration.

These conclusions were questioned by Levine (1919) who, as the result of inoculation experiments upon *Bryophyllum*, failed to obtain similar results either in the leaf notches or mid-veins. Later, Smith (1921) repeated his observations upon *Bryophyllum* and showed conclusively that not only were the axillary buds stimulated to growth by inoculation with *B. tumefaciens* below the petiole of the subtending leaf, but that the leaf-notch buds of young leaves responded similarly. More rarely, shoot-bearing tumours were produced by inoculation into the midrib of the leaf.

The exact nature of the stimulus was reserved for future discussion. It had been shown previously, however, that the chief indication of chemical change due to the presence of *B. tumefaciens* in the plant tissues is the production of an abundance of acetic acid at the expense of the stored carbohydrates; but whether the stimulus is chemical or not there is no doubt that a distinct crown-gall stimulus does exist for *Bryophyllum* although its effect is restricted to places where meristematic tissue occurs. As a consequence of this stimulus shoots are produced freely in the leaf notches whether the leaf is in connection with the plant or not. Smith's photographs leave no doubt whatever on this point and the possibility of the existence of a growth stimulus of a chemical nature for the buds of *Bryophyllum* cannot therefore be excluded.

Bryophyllum was regarded by Child and Bellamy (1920) as a favourable object of experiment for an investigation of the behaviour of single leaves or leaflets while in a state of physiological isolation. They started from the position that the dominance of a plant portion is associated with its metabolic activity seeing that the former is inhibited when the latter ceases, as when the dominant member is enclosed in plaster of Paris or in an atmosphere devoid of oxygen. They drew attention to similar relations between dominant and subordinate parts in the animal kingdom, which originate in differences in the rate of the fundamental reactions and are associated with differences in protoplasmic condition. Such relations appear in the form of a gradient in the physiological condition, in which the dominant part is primarily the region of greatest metabolic rate, *i.e.* of greatest physiological activity.

This relation between the dominant and its subordinate parts they considered to be primarily transmissive rather than transportative, dominance being dependent upon transmitted dynamic changes. In the less specialised protoplasm of plants they viewed the dynamic effect as undergoing a decrement with increasing distance from the point of origin, thus limiting the range of physiological dominance and creating a set of conditions where physiological isolation was possible.

They enumerated the following ways in which physiological isolation might be brought about.

(1) Growth, *i.e.* growth in size, might remove some part out of the range of the dominant.

(2) A decrease in the metabolic activity of the dominant might increase the range of the dynamic effect.

(3) The passage of the dynamic effect might be blocked.

(4) A subordinate part might be excited externally to counteract the action of the dominant which would therefore become inactive.

Physiological isolation in the leaf was achieved by surrounding the petiole with a coil of tin piping through which flowed a stream of cooled water at a temperature of from 2·5° to 6° C. The coil passed round 2–3 cm. of the petiole while the leaf-blade was submerged in water. Growth, in the form of one or more roots, appeared in the leaf notches three or four days after the coil was placed in position. If the coil was removed at this point few if any shoots appeared, but if it remained in position for six to eight days shoots appeared and continued to grow after the removal of the coil.

Separate experiments were carried out to show that the flow of water to the leaf was not stopped by the cooling of the petiole.

In every case all or nearly all of the buds in the notches of the cooled leaf produced vigorous shoots, while the opposite leaf showed a degree of growth almost as great. The leaflets of the nodes immediately above and below were similarly influenced, if they were submerged at the same time, but showed slighter development of the buds in the notches.

It was found that buds which failed to develop roots and shoots within a week made little or no further progress, being inhibited to a greater or lesser extent. These were regarded as weaker or less active buds which, reacting less rapidly than normal to the conditions of isolation, did not advance far enough before the return of normal conditions to maintain their growth afterwards.

The authors claimed that the cooled zone blocked the inhibiting

action of the chief growing tip and other parts without blocking the flow of fluids and substances in solution. They held that this was opposed to Loeb's assumption of inhibitory substances being transported through the usual channels, seeing that the only alternative is the inactivating of the inhibitor by the cooled zone. They advanced an alternative assumption, viz. that the dominance of the growing tip is due to some sort of action which is dependent upon the physiological activity of the cell. When this activity is blocked by the cooled zone the action is also blocked. Accommodation may be brought about and the temperature block become ineffective after a few days.

They preferred to regard physiological dominance as being dependent upon "some sort of effect transmitted physiologically through the living active protoplasm," rather than upon substances transported by the flow of liquids. Their criticism of Loeb's assumption is based upon the impossibility of reconciling a relationship whereby each part which produces such substances is immune to their action. This presumes a remarkable specificity of action on the one hand and absence of specificity on the other and Child and Bellamy found it difficult to conceive how the hypothetical substance could have the required properties. They held also that certain of Loeb's assumptions concerning the direction of flow of the inhibitory substances had no basis in fact and did not agree with the facts in hand. They pointed out, further, that Loeb appears not to distinguish clearly between the conditions that permit (or prevent) development in a subordinate part and the quantity of growth and development of this part subsequent to this initiation. The latter they explained is dependent upon the processes of nutrition, the former not.

Some difficulty may be found on trying to reconcile with the observed results the argument of Child and Bellamy that the so-called physiologically-isolated leaflet was affected beneficially by the blocking of the inhibitory effect of the chief growing tip. The effect of the cooling might equally well be interpreted as a stimulation radiating from the cooled zone, for not only did the opposite leaf appear to benefit but also those of the nodes above and below that from which the cooled leaf arose.

Nevertheless, in the absence of further and more convincing evidence, the theory of transmission of Child and Bellamy is more attractive from the physiological point of view than the theory of transportation supported by Loeb, Reed and Halma: both are, however, equally plausible.

It may, however, be pointed out that it is merely conventional to postulate an inhibitory action of the type described by the latter three workers. The observed phenomena might be equally well explained on the assumption of the production of a growth stimulator in the basal region and its transportation towards the apical region. Loeb himself held something of this nature to be the case before he abandoned the theory of a "suction of growth stimulating substances" for his theory of growth-inhibition.

It is difficult to avoid the impression that many of the workers on this portion of the subject have been influenced by certain phenomena of animal physiology to the extent of creating analogies which are incapable of being justified. In animal physiology inhibition takes place when a definite reaction to an external stimulus is brought to a standstill, the stimulus being conveyed along specially modified nerve tissue and the reaction being stopped by some action upon this conducting strand. In the plant the conditions for such a process do not exist for, although the whole of the protoplasm is capable of response to external stimuli, there are no specialised channels corresponding to nerve tissue for the purpose of conducting excitatory processes.

Furthermore, in the animal the specially differentiated circulatory system makes possible the transport to considerable distances of, for example, the haemolytic or haemotoxic substances produced by endoparasitic organisms. In the plant the movement of such substances would be confined either to the sap stream of the xylem or to the diffusion stream of the phloem and cortex.

Inhibition in the plant has always been applied to the stoppage of a vital process owing to the accumulation of substances which are the result of the process itself. But the proponents of the inhibition theory construct a different picture of the process in which the substance produced by the activity of a growing point, so far from being accumulated there, is transferred to a considerable distance rapidly enough to prevent the development of the growing points situated there. This not only presupposes a "remarkable specificity" as Child and Bellamy remark but demands special physical conditions in the plant which do not exist.

A further doubt may be raised as to the justification for the direct application to the woody shoot of the results obtained with a plant like *Bryophyllum*. In the detached nodes and leaves of the latter the water and nutrition relations must have departed considerably from the normal, while the amount of physiological response to factors

such as increased aeration at the wound surface must necessarily have been very great.

The production of an inhibitor by the terminal bud had previously been assumed by Erréra (1904) who was of the opinion that such a substance prevented the upward growth of the lateral branches of pines so long as the terminal was present. In this case also the hypothesis fitted the facts exactly.

As for the Hormone Theory of Geotropism it can only be said that here again hypothesis has too far outstripped experimental evidence. Evidence of activity in plants due to hormones is, to say the least, meagre. Moreover, for a final test of any theory of hormone activity, experiments on the lines of those of animal physiologists with extracts of the pancreas, thyroid and pituitary body will be necessary and hitherto no progress has been recorded in this direction.

Plant physiologists have, however, good reason to be grateful to Loeb, who has stimulated a measure of exploration of a neglected field and demonstrated several new facets of the problem of the development and growth of buds.

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(To be continued.)

THE EFFECT OF CARBON DIOXIDE ON THE TROPIC REACTIONS OF *HELIANTHUS* STEMS

By R. E. CHAPMAN, W. R. I. COOK and Miss N. L. THOMPSON

(With Plate I and 4 figures in the text)

This work began as an ordinary advanced class, routine experiment, but the results obtained were so contrary to Miss Lynn's(4) that the experiments were repeated with greater care; and, finally, owing to the theoretical interest of the subject, they were expanded into the present series, to test the behaviour of both normal and etiolated seedlings when grown in atmospheres containing different percentages of carbon dioxide.

Experimental Methods

Miss Lynn tried several plants in her experiments and obtained the best results with *Helianthus annuus*. In consequence, the whole of the following work was performed with *H. annuus* var. *Leviathan*.

Seeds were soaked in water and then germinated in garden soil in a roof greenhouse at King's College, or later, at the Chelsea Physic Gardens with the kind permission and help of the Curator, Mr W. Hales. The plants were divided into two series:

Series 1 Grown under normal conditions.

Series 2. Grown in darkness (etiolated).

In Series 1, after setting up the experiments, the plants were placed (*a*) in darkness, to study the effect of gravity alone, and (*b*) with side illumination, to study the effect of heliotropic as well as geotropic stimulus. In Series 2, the experiments were carried out entirely in darkness.

The seedlings were grown in sets of three per pot, and when 5–7 centimetres above the soil (except with some of the etiolated seedlings in Series 2, which grew to 12 centimetres) they were set up in bell-jars of 6·8 to 7·4 litres capacity, with the hypocotyls horizontal. The soil was thoroughly moistened to prevent wilting. The bell-jars were then closed by rubber stoppers, pierced by glass tubes, which were sealed by thick rubber pressure-tubing and glass

rods. The jars were sealed on to flat, ground-glass plates by means of vaseline or a mixture of beeswax and vaseline (see Plate I, fig. 5). Carbon dioxide (prepared from marble and commercial hydrochloric acid and then thoroughly washed by bubbling it through a strong solution of sodium bicarbonate to remove all traces of chlorine and gaseous hydrochloric acid) was then introduced to a predetermined concentration by displacement of water. The internal atmosphere was analysed for carbon dioxide at the beginning and, usually, again at the end of each experiment (and in some cases, once or twice during the experiment in addition) by means of a Hempel gas burette, special care being taken to fill with liquid the capillary tube between the upper tap of the burette and the absorption pipette, and thus avoid any error caused by the inclusion of air in the capillary tube. Usually the concentration of carbon dioxide decreased slightly in the 24 hours (which was the ordinary duration of an experiment), probably by the diffusion of CO_2 through the rubber tubing and stoppers. The variation in carbon dioxide is shown usually, as a probable error, in column 2 of the tabulated results.

In the experiments with darkened plants, the bell-jars were placed in a cupboard and examined at intervals. When subjected to light, they were put in diffuse daylight near a side window facing west, not over-shadowed by other buildings. They received light from one side only, in a plane perpendicular to the axis of the seedlings.

At the end of the experiments, pieces of the epidermis of the cotyledons were fixed, using Lloyd's absolute alcohol method (2), and the length and breadth of the stomatal aperture then measured with an eyepiece micrometer, in order to determine whether any relation existed between the concentration of the carbon dioxide and the degree of openness of the stomata. With such young plants, they were at all stages of development and they varied greatly in size, even when fully open. It was found, however, that the ratio $\frac{\text{length}}{\text{breadth}}$ gave a much more reliable indication of the degree of openness than did the actual area of the pore. When the stomata are widely open the ratio approaches unity; as they close the ratio increases. In order to get a reasonable probable error, usually about 30 stomata were measured in each experiment.

Finally, the plants were put into ordinary air and light, to determine whether the high carbon dioxide concentrations had or had not been toxic to the seedlings. In most cases, the bell-jar and its contents were photographed just before the removal of the plants.

No special measurement of the temperature inside the bell-jars was made. The room temperature by day was very constant during the period of the work, and is recorded in column 3 of the tabulated results.

The humidity inside the bell-jars was not measured, but must have been practically at saturation point, as shown by the condensed moisture inside.

The curvature of the hypocotyl was measured by means of a transparent protractor.

EXPERIMENTAL RESULTS

The results obtained are tabulated under the serial headings on pages 53, 54 and 57. Column 2 gives the average concentration of carbon dioxide during the experiment, column 3 the room temperature, column 4 the age of the seedlings at the beginning of the experiment, and column 5 the time from the commencement of the experiment (*i.e.* from passing in the carbon dioxide) at which the observation following in column 6 was made. This latter gives the movement of the seedlings in regard to the tropism under examination. The figure inside the bracket indicates the number of plants which showed the movement immediately following. Upward curvature is denoted by + and downward by −.

The probable errors of the stomatal ratios in column 7 were calculated from the formulae given by Mellor (5, pp. 524-9).

Series 1 a. *Normal seedlings in darkness* (see Table I)

In air and low percentages of carbon dioxide, the plants showed normal negative geotropism (see Plate I, figs. 1 and 2). The rate of response, as shown by the increased length of time necessary to reach any particular degree of curvature, decreased as the CO_2 content increased. In air (Exp. 12) the seedlings curved through 90° in 2 hours; in 7 per cent. carbon dioxide (Exp. 6) they took 4 hours to reach 90°; in 9½ per cent. carbon dioxide (Exp. 7) they required 5 hours, and in 16 per cent. carbon dioxide they had reached 90° in 23½ hours. In about 22 per cent. to 40 per cent. carbon dioxide they failed on the whole to reach 90°, even after more than 20 hours (Exps. 9, 10 and 13. Exp. 11 with 30 per cent. carbon dioxide was exceptional). At 44 per cent. and higher percentages of CO_2 (Exps. 13 *a* to 18) upward curvature almost ceased. Usually in these exceedingly high concentrations a slight downward droop was noticed. This was probably mechanical and due to the weight of the

TABLE I

Series 1 *a*. Normal Seedlings in darkness

Expt No.	% of CO_2	Temp. °C	Age (days)	Time (hrs.)	Curvature from horizontal. Upwards is + Downwards is −	Stomata. (Length / Breadth)	Remarks
12	air	22	8	1¼	(3) +curve	2·17±0·11	
				2	(3) +90°		
				18¾	(3) +90°		Considerable growth
6	6·86	21·8	7	1	(3) No change	2·15±0·14	
				2½	(3) Slight +curve		
				4	(3) +90°		
				20½	(3) +90°		
7	9·43	22	7	2	(2) +curve. (1) ±0°	1·84±0·12	
				5	(3) +90°		
8	16·2	21·8	7	1	(3) −droop	1·85±0·14	
				2¾	(3) ±0°		
				4¼	(2) +25°. (1) > +90°		
				23½	(2) +90°. (1) > +90°		
9	22·4	22	9	22	(3) +60°	2·12±0·12	
10	25·4	19·5	13	19	(3) +80°	2·68±0·10	
				20¼	(3) +80°		
11	29·9	21	7	1½	(3) +curve	2·16±0·10	
				20¾	(3) +90°		
13	40·1±0·95	21·8	7	18	(1) +50°. (1) +80°	1·55±0·11	Only two seedlings used
13*a*	44·4	20	8	18¾	(2) Slight −droop. (1) ±0°	—	
14*b*	48·4	19	14	1½	(3) Slight −droop	2·84±0·11	
				2½	(3) Slight +curve		Then put on klinostat: after 2¼ hours, no change
14*a*	49·6±0·26	22	12	5½	(3) Slight −droop	2·44±0·13	
				21¾	(3) ±0°		
14	50·8	22	7	3	(3) No change	—	
				20	(3) Slight −droop		Very young seedlings: put on klinostat; no change after 21½ hours more
15	54·9±0·47	22	8	1¼	(3) No change	1·58±0·06	
				2¼	(3) No change		
				21	(3) Slight −droop		
				22¼	(3) No change		
16	65·3	21	11	4	(3) No change	—	
17	76·4±0·25	21	7	1	(3) Slight +curve	1·91±0·10	
				19	(2) −droop. (1) ±0°		
				24¾	(3) No change		Put on klinostat: no change after 20 hours more
18	89·9±2·39	21·3	10	1¾	(3) No change	4·57±0·14	
				3½	(3) No change		
				22	(3) No change		

cotyledons. In about 50 per cent. CO_2 (Exps. 14 *b* and 14 *a*) the seedlings showed signs of recovery from this preliminary droop or sag, but in no case with more than 41 per cent. CO_2 did any plant show definite upward curvature (Plate I, fig. 4).

In all concentrations of carbon dioxide up to about 40 per cent. the plants were healthy and grew normally after removal from the bell-jars into ordinary air and light.

In some experiments the plants were placed on a horizontal klinostat in air after being taken out of the bell-jars. Unfortunately, several of these plants were spoiled, but in Exps. 14 and 17 (after 51 per cent. and 76 per cent. CO_2 respectively) the seedlings showed no change in position after 20 hours or more on the klinostat. Exp. 14 *b*, after 48 per cent. CO_2, gave a similar result after $2\frac{1}{4}$ hours on the klinostat. The very high CO_2 content had apparently acted as an anaesthetic and prevented the plant from receiving or at any rate recording any stimulus at all. There was certainly no signs of any "delayed response."

The stomatal ratios in this Series showed great variation (Table I, column 7); in 90 per cent. CO_2, however, the stomata were almost completely closed.

TABLE II

Series 1 *b*. Normal Seedlings in diffuse light. Side illumination

Expt No.	% of CO_2	Temp. °C.	Age (days)	Time (hrs.)	Geotropic curvature (vertical) + or − Heliotropic curvature (lateral)	Stomata. (Length/Breadth)	Remarks
31	air	21·5	12	$16\frac{1}{4}$	(1) +90°. No response to light (2) ±0°. 90° response to light	1·97±0·07	The seedling (1) which curved upwards was shaded by the other two. Light weaker on second day
				$40\frac{1}{2}$	(1) +80°. 10° towards light (2) +50°. 40° towards light		
25	9·78	21	10	1	(3) +curve	1·90±0·09	
				$19\frac{1}{2}$	(3) +90°. No response to light		
				22	(3) Definite light response		
				43	(2) 25° towards light (1) No response to light		
26	16·3±0·30	21	17	$1\frac{1}{2}$	(3) Slight+curve and response to light	—	
				4	(2) +60°. 30° towards light. (1) +50°		
				$6\frac{1}{2}$	(2) +90°. (1) +50°		Plant (1) not very healthy
				24	(3) +90°		
27	17·6±0·34	21	10	$\frac{1}{2}$	(3) +curve	2·47±0·08	
				20	(3) +90°. No light response		
				42	(3) Good light response		Vigorous growth
				45	(2) +90°. (1) +45°		
28	35·0±1·11	21	10	$\frac{1}{2}$	(3) No change	2·49±0·13	
				21	(3) +60°		
				$22\frac{1}{2}$	(3) +65°. No light response		
				$44\frac{1}{2}$	(3) No change		Yellow patches on leaves
30	59·2±1·3	21·5	12	$1\frac{3}{4}$	(3) Slight—droop	3·38±0·12	
				$17\frac{3}{4}$	(3) Very slight recovery		
				$41\frac{1}{2}$	(3) No change		

Series 1 b. *Normal seedlings with side illumination* (see Table II)

This Series was made to determine whether increased CO_2 affected both heliotropic and geotropic response in the same way. The experiments were set up usually about 4 p.m. in July, 1923 (bright, sunny weather).

In air and low concentrations of carbon dioxide, both normal heliotropic and geotropic response were shown. Except in the control in air, the reaction to gravity was rather more pronounced and preceded that due to light (see text-figs. 1, 2 and 3). In the seedlings grown in air (Exp. 31), simultaneous response to light and gravity might have been anticipated, but, as shown by text-figs. 1 and 2, the reaction to light was completed before the response to gravity began. In 10 per cent. CO_2 (Exp. 25, text-fig. 3) the seedlings had reacted 90° to gravity in $19\frac{1}{4}$ hours, but without responding at all to light; this came $2\frac{3}{4}$ hours later. In 16 per cent. CO_2 (Exp. 26) both responses appeared together. Exp. 27 with $17\frac{1}{2}$ per cent. CO_2 showed after 20 hours 90° response to gravity but none to light; the response to light followed later. In 35 per cent. CO_2 (Exp. 28, text-fig. 4), after $20\frac{1}{2}$ hours the plants had reacted 60° to gravity but not to light; even after $44\frac{1}{2}$ hours, no heliotropic response was seen. Exp. 30 with 59 per cent. CO_2 showed no upward curvature at all, even after $41\frac{1}{2}$ hours, and no heliotropic response. These results agree with Series 1 *a*; as the concentration of carbon dioxide increased, the rate of response slowed down and ultimately ceased.

An interesting point which emerges from the results is the tendency for geotropic curvature to take place first in the lower percentages of carbon dioxide (about 10 per cent.), whilst in air, the heliotropic response took place first. With higher concentrations of carbon dioxide, the heliotropic curvature seems to be inhibited altogether, though a definite geotropic response occurs (Exps. 27 and 28). On the other hand, Exp. 26 with 16 per cent. CO_2 differed from Exp. 27 with 17 per cent. in that both tropisms had definitely appeared after $1\frac{1}{2}$ hours. This seems to be a subject worthy of further investigation.

The stomatal readings in this series showed a definite and progressive closing as the CO_2 content increased, the $\frac{\text{length}}{\text{breadth}}$ ratio ranging from 1·97 in air to 3·38 in 59 per cent CO_2.

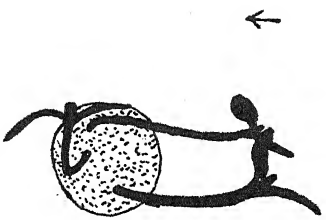

Fig. 1

Fig. 2

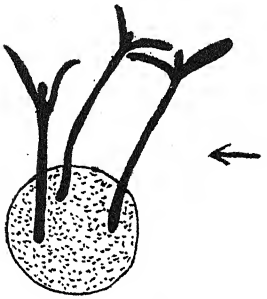

Fig. 3

Fig. 4

Series 1 *b*. Normal seedlings with side illumination. (The arrow shows the direction of the light.)

Fig. 1. Exp. 31. Control in air. Photographed after 20½ hours.

Fig. 2. Exp. 31. Same plants as in Fig. 1, photographed after 45½ hours. They now show complete heliotropic and geotropic response. The left seedling was shaded by the other two, and so reacted first to gravity (Fig. 1) and then to light (Fig. 2).

Fig. 3. Exp. 25. In 10 per cent. CO_2. Photographed after 47 hours. Showed 90° response to gravity in 19 hours, followed by heliotropic response of two seedlings 3 hours later.

Fig. 4. Exp. 28. In 35 per cent. CO_2. Photographed after 46½ hours. (The direction of the light in this experiment was perpendicular to the paper.) Shows no heliotropic response at all, but 60° response to gravity.

(Text-figs. 1–4 were prepared from bromide prints of the photographs. The plants were marked in in Indian ink and the print then bleached.)

Series 2 a *Etiolated seedlings in darkness* (see Table III)

This Series was made in September, with rather lower air temperatures of 16° to 19° C. (except Exps. 21–24, which were made in August).

The results are similar to those obtained in Series 1 *a*. With low percentages of carbon dioxide the response to gravity was complete,

TABLE III. Series 2 *a*. Etiolated Seedlings in darkness

Expt. No.	% of CO_2	Temp. °C	Age (days)	Time (hrs.)	Curvature from horizontal Upwards is +. Downwards −	Stomata. (Length / Breadth)	Remarks and length of seedlings
24	air	19	8	1¾	(3) −20°	—	*c.* 12 cm. long
				2¾	(3) +55°		
				26	(1) +90°. (1) +80°. (1) +65°		
32	air	15	9	½	(2) ±0°. (1) +10°	1·77±0·10	*c.* 6–7 cm. long
				2	(3) +20°		
				18½	(3) +90°		
				42½	(3) +90°		
21	11·4±0·79	19	8	1	(3) −25°	—	*c.* 12 cm. long
				2	(1) Slightly +. (2) ±0°		
				25	(2) +90°. (1) +60°		
33	13·3±0·61	16	11	½	(2) −10° (long). (1) ±0°	1·38±0·07	Two *c.* 12 cm. long
				18¾	(2) +60°. (1) +90°		One *c.* 6 cm. long
34	14·2±0·29	18	5*	½	(3) −15°	1·72±0·04	*c.* 7 cm. long
				17	(3) +70°		
				19½	(3) No change		
				24½	(3) No change		
				41	(2) +70°. (1) +75°		
				43	(3) No change		
35	18·2±0·48	18	5*	16½	(3) +65°	1·97±0·06	*c.* 5 cm. long
				18¾	(3) No change		
				24	(3) No change		
				40½	(2) +65°. (1) +90°		
22	20·4±1·03	19	8	¾	(3) −30°	—	*c.* 12 cm. long
				1¾	(3) −40°		
				25¼	(1) +80°. (1) +40°. (1) +15°		
36*a*	27·2±0·39	18	5*	½	(3) −10°	2·74±0·12	*c.* 5 cm. long
				1	(3) −20°		
				17½	(2) +60°. (1) +30°		
				25	(2) +60°. (1) +30°		
				41½	(2) +70°. (1) +35°		
				65½	(2) +70°. (1) +40°		
				91	(1) +70°. (1) +50°. (1) +40°		
23	± 30·1 −0·44	19	8	½	(3) −30°	—	*c.* 12 cm. long
				1¾	(3) −60°		
				25	(2) −80°. (1) +60°		Exp. had then to be stopped
36	30·6±0·14	15	10	½	(2) −10°. (1) ±0°	2·76±0·20	*c.* 6 cm. long
				1¼	(2) −25°. (1) ±0°		
				2¼	(2) −30°. (1) −5°		
		18		21	(1) +40°. (2) +60°		
37	32·5±0·79	16	7*	1	(3) Slight−droop	2·84±0·14	*c.* 6 cm. long
				3	(3) No change		
				4	(3) No change		
				21	(2) +45°. (1) +70°		
				47	(2) +50°. (1) +80°		
37*a*	37·7±0·61	16	7*	1½	(3) Slight−droop	2·13±0·25	*c.* 6 cm. long. Stomatal measurements very difficult, because the cotyledons were so young
				2½	(3) No change		
				19½	(3) +70°		
38	58·1±1·21	19	6*	2	(3) Slight−droop	2·61±0·15	*c.* 6 cm. long
				16½	(3) ±0°. (1) −5°		

The mark * in column 4 indicates germination at a higher temperature than usual.

but steadily diminished and ultimately ceased as the concentration increased. Exp. 24 in air showed a movement of + 75° in one hour, a rate of response not approached by any plants of this Series in any other atmosphere, so far as the records go. In 11 per cent. CO_2 (Exp. 21) the seedlings moved through about 30° in one hour, whilst from 13 per cent. (Exp. 33) with a rise of 70° in 18¼ hours, to 38 per cent. (Exp. 37 *a*) with a rise of about 75° in 18 hours, there was very little difference in the rate of response to gravity. The single experiment at 58 per cent. CO_2 (Exp. 38) showed very little response after 19 hours, a result which is in agreement with the other Series, where concentrations higher than about 40 per cent. stopped all movement of the seedlings. As regards the final position of the seedlings, only in Exp. 32 in air with short seedlings did all the three plants reach + 90°; in the other control in air (Exp. 24) and in 11 per cent. (Exp. 21) two plants reached the vertical; and in 13 per cent. and 18 per cent. (Exps. 33 and 35) one seedling in each reached + 90°. In 27 per cent. CO_2 (Exp. 36 *a*), though continued for 91 hours, the plants only reached + 70°. This final degree of curvature was maintained up to 38 per cent., but in 58 per cent., as already noted, all curvature ceased.

The experiments with the longer and therefore mechanically weaker seedlings showed a considerable droop or sag, even in air (Exps. 24, 21, 33, 22 and 23). Exp. 33 in 13·3 per cent. CO_2 is very interesting as there were two sizes of seedlings in the one pot. The two longer ones showed a marked preliminary droop, whereas the shorter one never drooped at all. All three showed strong normal geotropism.

Exp. 23 in 30 per cent. CO_2 seems at first to support Small and Lynn's theory, that high concentrations of carbon dioxide cause abnormal positive geotropism, but even here, although after 1¾ hours all the seedlings were − 60°, yet after 25 hours one had recovered to + 60°, though the other two had drooped further to − 80°. Unfortunately, this very interesting experiment had then to be stopped. Other experiments with concentrations very near to 30 per cent. CO_2 (Exps. 36 *a*, 36 and 37 with 27 per cent., 31 per cent. and 32½ per cent. respectively), but with short seedlings, all gave a slight preliminary droop followed by very marked normal geotropism.

DISCUSSION OF RESULTS

Small and his co-workers (7, 8, 9 and 4) state that high concentrations of carbon dioxide reverse both the geotropic and heliotropic responses of the stem. Our experiments not only did not

support this view but gave directly contrary results. None of our results, save the solitary Exp. 23 (and only two out of three seedlings there), showed permanent downward curvature or anything which could be described as positive geotropism, such as must be produced if his hydrion theory of geotropism is sound.

Lynn (4) usually fails to give certain essential details, particularly the history of the seedlings prior to the experiments, and the exact length of time in which they were under abnormal concentrations of carbon dioxide. In one case (her Exp. 1, p. 119) a day in 33 per cent. of CO_2 may be assumed, and again in one case only (her Exp. 20, p. 120) does she specifically state "these seedlings had been brought on in bright light." Her photographic illustrations would seem strongly to suggest that usually the seedlings were etiolated. This impression is confirmed by comparing the long white stems of her Plate I, figs. 1–4, with our Plate I, figs. 1–4 (of non-etiolated seedlings) and Plate I, figs. 5–9 (of etiolated seedlings). Our entire set of experiments leads to the conclusion that Lynn's "downward curvature" in high concentrations of carbon dioxide is solely mechanical and not geotropic, and that had she kept her seedlings in the CO_2 atmospheres for longer periods, she would have obtained results exactly similar to those produced by putting the plants into air. Hence her explanation (p. 121) that the "reversal of geotropic curvature actually does occur...in an atmosphere containing from about 9 per cent. to 30 per cent. of carbon dioxide" is based on insufficient data and cannot be accepted.

Our Exp. 33 (see Plate I, fig. 6) with etiolated seedlings after 19 hours in 13·3 per cent. CO_2 showed two seedlings with exactly the same "downward curvature" and subsequent recovery as Lynn's Exp. 15 (see her Figs. 3–4), also in 13·3 per cent. CO_2, though in her case the subsequent upward recovery was obtained after removal to air and is therefore ascribed by her to the change of atmosphere. The third seedling of our experiment was much shorter than the other two and therefore mechanically stronger, and definitely does not show the downward curvature. Our other experiments with long, etiolated seedlings invariably gave the same preliminary drooping.

It is well known that etiolated seedlings have less strength and elasticity than normal ones, and also that any curvature produced by an external force can be fixed by later growth (see Plate I, fig. 6, where the mechanical droop has been fixed by growth and then followed by normal geotropism).

Our experiments with plants placed on a horizontal klinostat in air after being kept in high concentrations of carbon dioxide, were made in order to test whether the plants had received a geotropic stimulus, but, owing to inhibition of growth by the high percentage of CO_2, they were unable to respond. No evidence of such delayed response was found. Lynn's experiments with plants on a klinostat and still in a high percentage of carbon dioxide also showed no response by the seedlings. This she interpreted as proof that her "downward curvature" of stationary plants in high CO_2 concentrations *must* be geotropic. Whatever force may be the cause of the "downward curvature," a revolving klinostat would continually change the direction of the force, whether mechanical or gravitational, and in any case therefore there should be no response.

The stomatal ratio $\frac{\text{length}}{\text{breadth}}$ fluctuated in Series 1 *a*, but gave definite progressive closing in Series 1 *b* and Series 2 *a*. This is in accordance with the results obtained by other workers. In 1876 Burgerstein (1) found that weak aqueous solutions of acids, including carbonic acid, when used in water cultures of different plants caused increased transpiration. Pfeffer (6, vol. 1, p. 230) states that "a high percentage of carbon dioxide in the gases of the soil or culture fluid causes a diminution in the rate of absorption" of water. Loftfield (3) and other workers have conclusively shown that the stomatal aperture varies directly with the available water present in the plant; an atmosphere containing a high percentage of carbon dioxide would therefore tend to cause the stomata to close by increasing transpiration and decreasing the rate of absorption.

Our thanks are due to Professor R. R. Gates for many useful suggestions and criticisms.

SUMMARY OF RESULTS

1. Both normal and etiolated seedlings of *Helianthus annuus* grown in concentrations of carbon dioxide up to 40–45 per cent. show normal geotropic response.

2. Normal seedlings of *H. annuus* grown in concentrations of carbon dioxide up to 40–45 per cent. show normal heliotropic response.

3. Above 40–45 per cent. of carbon dioxide, growth ceases and with it all response to gravity and light.

4. The preliminary downward curvature of etiolated seedlings in all concentrations of carbon dioxide where any downward move-

ment is visible, is due to mechanical stress and not to a reversal of geotropism.

5. In the higher concentrations of carbon dioxide the geotropic response usually occurs first, followed by the heliotropic response if the latter takes place at all.

6. With an increase in the concentration of carbon dioxide there is a general tendency for the stomata to close.

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Since the above was written a paper has been published by N. Cholodny ("Zur Frage nach der Rolle der Ionen bei geotropischen Bewegungen," *Ber. d. deut. Bot. Gesell.* **41**, pp. 300–311, 1923), who has been working on the same problem using lupin and *Helianthus* seedlings in usually about 20 per cent. carbon dioxide.

He comes to much the same conclusions as we do with regard to Small and Lynn's interpretation of their results. In his discussion, he writes that his results agree with Lynn in that after an hour or less, etiolated *Helianthus* seedlings were clearly observed to be directed downwards, but goes on to state that the bending angle was relatively small and the bending was so distributed along the stem that the natural explanation was that it was due to a passive sinking down of the seedlings. Many seedlings showed normal geotropic

reaction. He states further that in Lynn's experiments the photographs show no normal downward curvature, and only in long seedlings do the figures show downward bending at all. Small's experiments with roots and stems prove nothing. The curvatures are the result of pathological or abnormal physiological changes caused by the reagents used.

Cholodny's own experiments showed that *Helianthus* stems bend downwards in about 20 per cent. carbon dioxide, and that afterwards this downward bending becomes fixed by growth. Negative geotropism was never observed in roots nor positive geotropism in stems. He concludes that the origin of such abnormal bendings was connected with the effect of the carbon dioxide, which altered the mechanical properties of the stem. He explains this result as not being a geotropic reaction at all, but the passive result of the weight of the cotyledons, the stem becoming less elastic and more plastic. He tried to prove this experimentally by attaching small weights behind the cotyledons, and states that the plasticity of the stem increases to more than double after one hour in about 20 per cent. carbon dioxide.

EXPLANATION OF PLATE I

Figs. 1–4 refer to Series 1*a* (normal seedlings in darkness).

Fig. 1. Exp. 12. Control in air. Photographed after 19 hours.

Fig. 2. Exp. 7. In 9 per cent. CO_2. Photographed after 5 hours.

Fig. 3. Exp. 13. In 40 per cent. CO_2. (Only two seedlings.) Photographed after 17 hours. Shows slow but normal geotropic response, retarded by the high concentration.

Fig. 4. Exp. 15. In 55 per cent. CO_2. Photographed after $22\frac{1}{2}$ hours. Shows no response at all, presumably owing to cessation of growth.

Figs. 5–9 refer to Series 2*a* (etiolated seedlings in darkness).

Fig. 5. Exp. 32. Control in air. Photographed after $17\frac{1}{2}$ hours. Shows method of support, allowing the seedlings free movement.

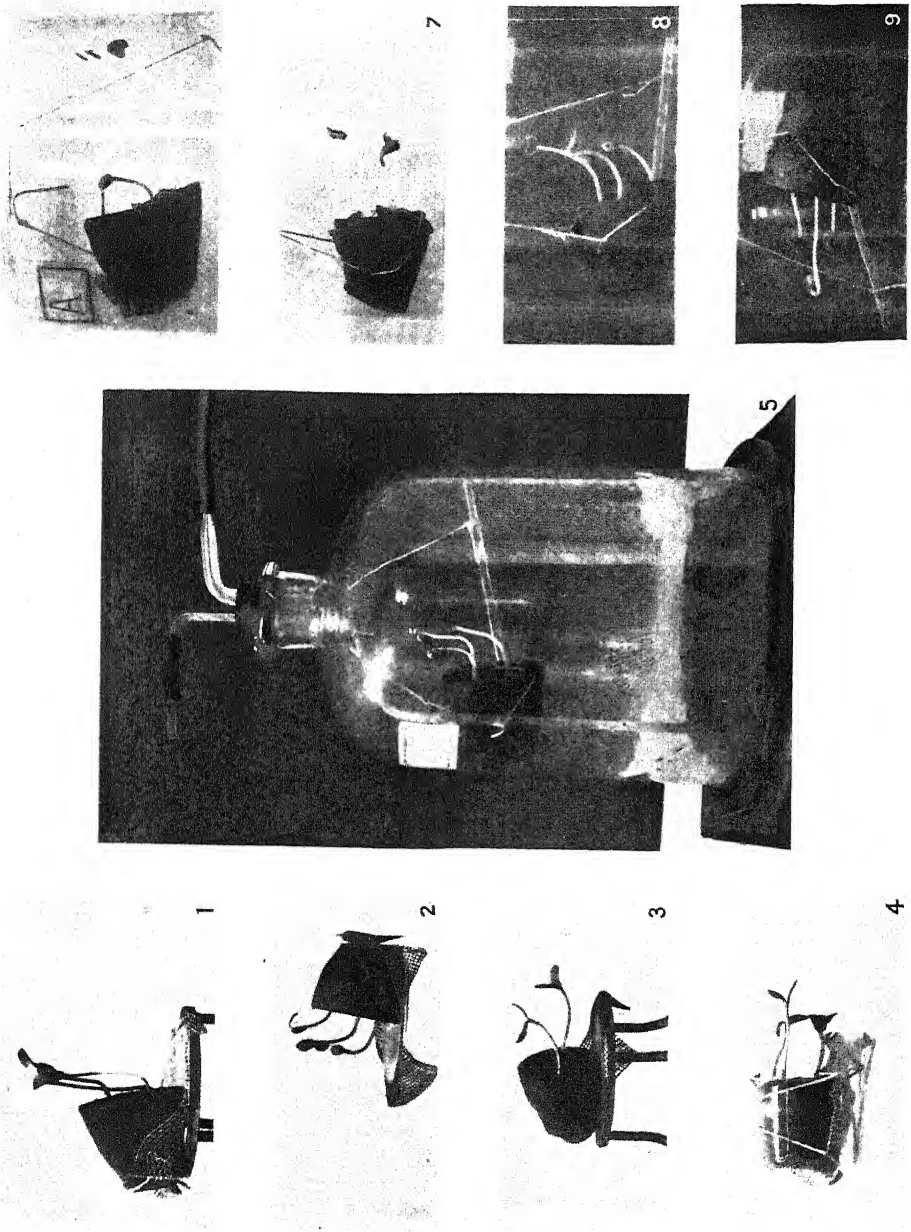
Fig. 6. Exp. 33. In 13 per cent. CO_2. Photographed after 19 hours. One short seedling shows normal geotropism: two longer seedlings show the preliminary droop and ultimate normal response.

Fig. 7. Exp. 36. In 31 per cent. CO_2. Photographed after $26\frac{1}{2}$ hours.

Fig. 8. Exp. 37. In 32 per cent. CO_2. Photographed after 20 hours.

Fig. 9. Exp. 38. In 58 per cent. CO_2. Photographed after $21\frac{1}{2}$ hours.

(Compare Figs. 5–9 with Figs. 1–4.)



EFFECTS OF CARBON DIOXIDE ON TROPIC REACTIONS IN HELIANTHUS

REVIEWS

Two Books on Fungi

A Handbook of the Larger British Fungi, by John Ramsbottom. London. Printed by order of the Trustees of the British Museum. Price 7*s.* 6*d.*

This Handbook, which is based on Worthington G. Smith's *Guide to Sowerby's models of British Fungi in the Natural History Museum, South Kensington*, is essentially a new book, and it should serve as an extremely useful introduction to the study of the larger fungi. When first collecting Agarics and Polypores beginners are usually bewildered in attempting to differentiate the multitude of forms described in the larger books; Mr Ramsbottom's Handbook should remove this difficulty as attention has been confined to the well-known species illustrated by Sowerby's models together with certain other common types, the descriptions being limited to essential characters. Special emphasis is laid upon the determination of genera, which in this edition include all the higher members of the British Basidiomycetes.

The classification adopted in the Handbook is that used in the display of Sowerby's models, and for this reason no attempt has been made to express modern views on the classification of certain groups, as, for example, those represented in Rea's *British Basidiomycetae*. This was doubtless inevitable at present, but, for the sake of students, it may be hoped that on a future occasion the classification may be brought into line with recent views. Such an anomaly, for instance, as that of including *Exobasidium* in the Thelephoraceae should not continue.

The Handbook opens with an account of the characteristic features of fungi, followed by particularly interesting paragraphs upon some of the most attractive relationships of these organisms, *e.g.* fairy rings, mycorrhiza, colour changes, esculent and poisonous properties, etc. Throughout the systematic part of the book also the author gives a mass of information about the economic importance of fungi, the accounts of mushroom cultivation and of "dry rot" of structural timber being especially lucid. The matter is presented in very readable form, and there are clear illustrations of some of the chief genera. Further details of Buller's fascinating work on the organisation of the hymenium of the Agaricaceae might perhaps have been included, and it would have been advantageous if references to recent literature could have been cited, especially as the authors' names are usually given. A few minor comments may be made: although *Fomes annosus* is described as the chief cause of heart rot of conifers in this country no mention is made of its root parasitism, and under *Polyporus squamosus* the author states that "affected wood shews a black line similar to that of *Armillaria mellea*," whereas this fungus typically causes a white rot, the "black lines" being frequently absent.

Mr Ramsbottom and the authorities of the British Museum are to be heartily congratulated upon the appearance of this valuable Handbook, for, as has been said elsewhere, the writer has provided therein "a tale of the exciting adventures of toadstools and cup fungi which will not soon be exhausted." The book will be specially welcome to students.

F. T. BROOKS.

Diseases of Crop-plants in the Lesser Antilles, by WILLIAM NOWELL, D.I.C., with a foreword by Professor J. B. FARMER, F.R.S. Published on behalf of the Imperial Department of Agriculture by the West India Committee. Pp. xix + 383. Price 12*s.* 6*d.* net.

This book is a welcome addition to the few books at present available on the diseases of crop-plants in the tropics. Although the book treats particularly of the cultivated plants in certan West Indian islands it is of widespread value as much of the information contained in it is applicable to other regions of the tropics. Owing to the diversity of crops grown in the West Indies there are few tropical plants of economic importance which are not mentioned in these pages.

The book is divided into two parts. The first deals with the general principles of plant pathology, with the nature of plant diseases caused respectively by fungi, bacteria, viruses, nematodes, etc., and with the prevention and control of these diseases by the application of fungicides and by other measures. In the first chapter the author introduces a section on "Notable groups concerned in fungus diseases," in which the general characteristics of such heterogeneous collections of fungi as the rusts, the anthracnoses, the rhizoctonias, the diplodias, the genus *Marasmius*, etc., are discussed. This is an innovation in the description of plant diseases, which is of doubtful value. The information in this section is also contained in other parts of the book; it is of no particular interest to the plant pathologist and is not likely to appeal to the practical cultivator.

The second part of the book is much larger and is by far the most interesting and valuable. It deals with the diseases of such important crop-plants as cacao, coconut, banana, cotton, and sugar-cane among others, especially from the point of view of one who, as the author says in the preface, from being a mycologist has had to become an agriculturist. With this refreshing outlook the writer emphasises the importance of soil and other environmental conditions upon the incidence of certain diseases, and the book is full of illustrations of the inter-relations of host, parasite and environment in the causation of disease. Many fungi thus fall from their "high estate" as destructive parasites, a certain condition of weather or soil being the real factor which leads to opportunity for the pathogen to do its destructive work. In this connection the pages devoted to root disease of sugar-cane are particularly illuminating, and it is this outlook, which needs to be more and more emphasised, that gives the book its special value.

Most of these diseases of crop-plants are described at length, but sometimes the details are needlessly profuse. The text is well illustrated and there is an excellent bibliography. The value of the book is enhanced by a notable "Foreword" by Professor Farmer, who writes of the necessity of a broad outlook upon the problems of disease in plants, such as is inculcated in this book by his former student.

The Imperial Department of Agriculture of the West Indies and the West India Committee have published a book which fills a gap in the literature of plant pathology in a most successful manner.

F. T. BROOKS.

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ON THE EARLY GROWTH RATE OF THE INDIVIDUAL FUNGUS HYPHA

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(With two graphs and three tables in the text)

IT has repeatedly been shown (*e.g.* by Penfold(1)) that whe bacteria from an old culture are inoculated into fresh medium, the inoculated organisms develop at a rate which is not constant but continuously rises, at least for a time. If, for example, 100 bacilli are inoculated and become 200 in 40 minutes, the 200 will become 400 in perhaps 35 minutes, the 400 become 800 in 31 minutes, the 800 become 1600 in 28 minutes, and so on, each successive generation arising in less time than that which precedes it, until a minimum period is reached, which thereafter remains constant for a long time. Acceleration of this kind has been described in the case of many growing organisms, and a certain amount of evidence brought forward that in the course of the development some substance is formed which favours the growth.

It has also been found by many authors, *e.g.* by Fawcett(2) and Brown(3), that in its early stages of development a fungal culture grows more and more quickly as time goes on. In successive equal intervals of time a progressively larger increment of growth is made, over a period whose duration depends on the medium, the temperature and the particular fungus examined, but usually continues for several hours and often for several days. In this respect, then, fungi might seem to resemble bacteria. The investigations on fungi, however, which show this phenomenon, have mostly been made by the method of measuring at frequent intervals the diameters of colonies spreading on solid media. This is a convenient, and for many purposes certainly the best available method. But it is hardly possible to determine by it whether a true increase in the rate of growth is taking place. It is evident that (just as in the case of a sum of

money accumulating at compound interest) larger and larger total increments will be made in equal times, if the amount of growing substance is also increasing, although the growth rate really remains constant; and no sound estimate of the amount of growing substance is possible by the colony method. It was hoped that a study of the behaviour of the individual growing hypha might help to clear up this and other points, and the present paper contains the results of an investigation of the early growth rate of the single hypha.

The organism examined was *Botrytis cinerea*, which is particularly suitable for this purpose, as it shows little tendency to staling. The strain used was that described in previous papers (4, 5). The cultures were all descended from a single spore, were maintained always on Czapek's agar, and were always of about the same age, 4–6 weeks, at the time of use. Miniature agar plates were made of Coons' agar (for method see (6)), inoculated with a small number of spores and inverted over glass cells, which were provided with open lateral tubes and were filled to a constant depth with Coons' fluid. They were then incubated at 24°–25° in vessels containing a layer of distilled water, and after germination had occurred were transferred to the stage of the microscope, which was enclosed in an electrically heated constant-temperature box. The developing cells were easily visible through the agar, the growth of the hyphae could be readily followed, and was measured from time to time by means of an eyepiece micrometer. Light was admitted to the mirror through a window, which was kept covered except when measurements were being made. Each measurement occupied a very short time, usually less than a minute; and the temperature within the box remained steady within one degree, provided the room temperature did not vary greatly during the time of the experiment.

The results were at first bewildering in their apparent irregularity. All the hyphae were growing at different rates. For example, one hypha might increase in length by 42μ, while another was growing 30μ, a third 26μ and a fourth and fifth 10 and 11μ; and this although they were all on the same preparation, distant from one another not more than a couple of millimetres, and under conditions of growth as identical as one could hope to achieve. Further no one of these hyphae, taken by itself, maintained steadily its own rate but each was continually altering—*e.g.* it might grow 42μ in 30 minutes at one time and 72μ in the same period at another time, no change of temperature having occurred in the interval. Similarity of environment appeared to produce no similarity of result, even with

spore material as homogeneous as could be obtained. Many of these inconsistencies still remain unexplained, and there certainly appear to be great individual differences between one hypha and another; but out of the mass of data certain facts have emerged which bring some degree of order into the phenomena.

It soon became apparent that growth takes place only at the tip of the hypha. The hypha as it lengthens lays down behind the apex septa which divide it into cells or segments. It is evident from simple inspection that most of the growing is done at the apex, but it was reasonable to expect that a certain amount of elongation would occur in the segments also for some time after they were formed, and that the total elongation would be made up of a number of partial increments contributed by the individual segments and the apex. Investigation, however, showed that this was not the case. Extension takes place exclusively at the tip and a segment once formed does not elongate further. This is not a peculiarity of *Botrytis*, but is the usual method of elongation found in fungi (6).

The establishment of this fact greatly simplified the process of measuring and reduced the time required, since at each measurement all that is required is to determine the distance between the tip and some easily recognisable point on the hypha, whose distance from the spore has been previously ascertained.

In each hypha the rate at which the tip advances is not constant, but goes through a series of changes. At first, when the hypha is very short, the elongation is very slow indeed (*e.g.* 0·1 or 0·2μ per minute), but the rate gradually increases as time goes on and the hypha becomes longer, until eventually it may be 20 or 30 times as great as at the first measurement. A typical example is shown in Table I and Graph I. A single spore was sown, and after 6½ hours' incubation at 24°–25° C. the preparation was transferred to the microscope stage at 21·5°, and half-an-hour later the first measurement made. The tube then measured 30μ; 54 minutes later it measured 37·5μ, a growth rate of 0·14μ per minute. The rate steadily increased until, between 48 and 49 hours after sowing, it had reached the value of 3·34μ per minute. At this rate it remained fairly constant for about 9 hours, and then slowed down, and by 94 hours growth had practically ceased. By this time the tip had almost reached the edge of the agar platform on which it was growing. Further, the agar surface was inconveniently crowded by the development of branches from the original hypha. Their presence must have affected the conditions under which the hypha was growing, and it

is therefore not certain that the fall in rate would have occurred under other circumstances. This branch-growth is always troublesome, and if more than four or five spores are growing on the agar, it may become so profuse in 24 hours as to make further observation valueless and perhaps impossible.

TABLE I

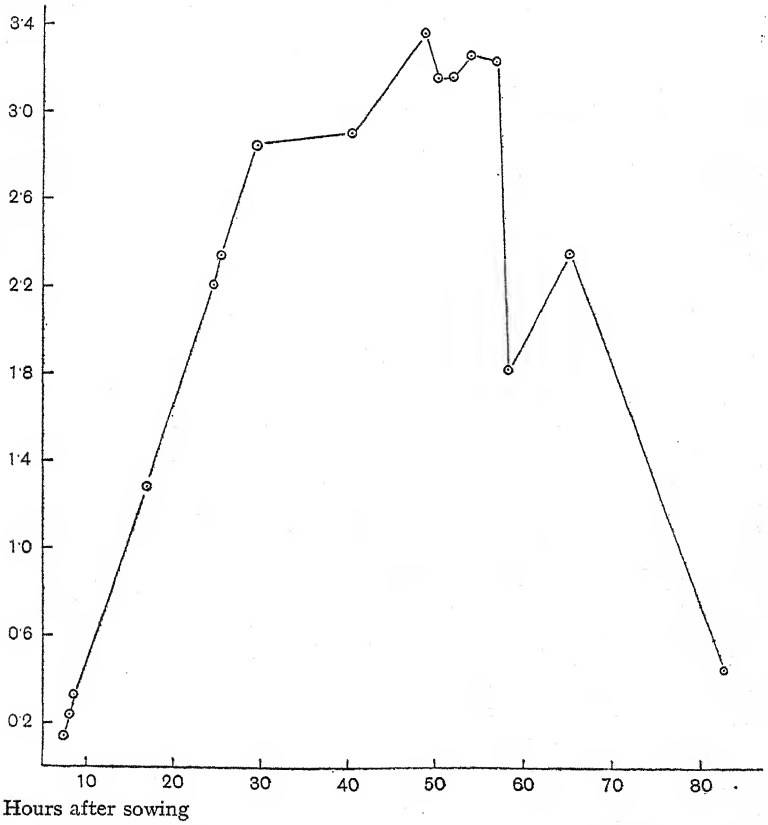
Time after sowing h.	m.	Length of hypha in millimetres	Rate in μ per minute
6	56	0·0300	—
7	50	0·0375	0·14
8	21	0·0450	0·24
8	54	0·0558	0·33
24	17	1·2441	1·28
25	22	1·3875	2·21
26	42	1·5750	2·34
32	51	2·6241	2·84
48	15	5·2947	2·90*
49	37	5·5691	3·34
50	41	5·7700	3·14
53	3	6·2175	3·15
55	8	6·6225	3·24
56	7	6·9083	4·84
57	1	7·0817	3·21
59	37	7·3641	1·81
70	55	8·9541	2·34
94	50	9·5466	0·45

* During the night the temperature fell to 19°; this figure is therefore too low.

Such an increase in rate is the general rule. Out of 34 consecutive unselected experiments, carried out at different temperatures and maintained for 6–8 hours, the growth rate, determined at intervals of 2–4 hours, increased throughout in 26, or 76 per cent. of the whole. The curve on plotting is not always so smooth as in Graph I, and the increase is not always uniform, but it is continuous. In about one-fourth of the cases, however, this is not the case. In 9 per cent. of the experiments the rate fell continuously, and in 15 per cent. it rose at first and then fell later. Almost all conceivable variations occur occasionally. A hypha may grow vigorously and at increasing rate for some hours and then slow down, gradually or abruptly, although other hyphae alongside it continue the normal course. It may even stop entirely, and instances have been observed where growth has ceased for a time and then been resumed. No explanation of these irregular, and on the whole infrequent, occurrences has been found. In no case was any definite connection observed between the rate of growth of the hypha and the laying down of septa.

The development of branches followed the same general course.

A branch may arise immediately behind the growing tip and keep pace more or less with the development of the main stem. Or it may arise from a segment far back in the hypha, several hours after the segment has been formed, and separated from the main tip by 20 or 30 segments (which in this strain and on this medium average



Graph I. Showing change of rate in extension of hypha at different times (see Table I). Observations plotted at middle of the time-intervals to which they refer. Growth at 21·5° C.

about 50μ in length); and it may do so although several other branches have already appeared between it and the main tip. Two branches may arise from the same segment, although in such a case it is usual for a septum to appear later between the two. The new centres of growth apparently arise and develop independently of the growing point of the original hypha (but see pp. 72–77 *infra*).

As in the parent hypha, the branch usually started slowly and increased in rate as time passed, though here again the same irregularities occurred as in the main stem. Normally the first branch did not appear till the main hypha had been growing for some hours and had attained a size of 0·3 millimetre or more, by which time its rate had considerably increased. It is a nearly constant rule that during the first few hours of its development the branch adds less to its length than the parent does in the same hours. It may eventually attain the same speed as the parent, but it starts more slowly. If, however, one compares the rate of growth of a branch of given length with the rate at which its parent grew when it was of the same length, one finds that in most of the cases (over 70 per cent.) the branch grows faster than its parent did. This fact has some bearing on the theory that the growing hypha gives off into the medium some substance accelerating its own growth (*v.* p. 77).

On the same preparation the growth rate of different hyphae shows some variation. As we have seen, some slow down or stop entirely, but even when we neglect these aberrant cases there is variation amongst those which continue to grow freely. This, however, is not so great as appears at first sight. Since the rate of growth increases as the hypha develops, it is evident that the longer the hypha the greater will be the speed at which it is growing; and in comparing the rates of different hyphae this fact must be taken into account. The spores sown on the agar plates germinate at different times, and when measurements are begun, the hyphae found are already of different lengths, and are consequently growing at different rates, even when perfectly normal. Comparison can be properly made only between the times taken by the hyphae to increase from one particular limit of length, *e.g.* 150μ, to another limit, *e.g.* 500μ. When this is done, most of the differences which are at first so confusing disappear. The results obtained are best shown by some examples. These are from eight consecutive experiments, and all hyphae measured are included.

(*a*) Five hyphae, each from a different spore, measured on one preparation. In growing from 160 to 600μ the rates of the five were 1·01, 1·18, 1·08, 1·15 and 1·02μ per minute (23°).

(*b*) Four hyphae measured; growth from 160 to 2600μ; rates 1·62, 1·66, 1·69, 1·75μ per minute (25°).

(*c*) Four hyphae measured; growth from 220 to 1100μ; rates 1·59, 1·26, 1·49 and 1·43μ per minute (22·5°).

(*d*) Four hyphae measured; growth from 220 to 600μ; rates 0·95, 0·81, 0·96, 0·91μ per minute (18·5°).

(*e*) Five hyphae measured; growth from 240 to 360μ; rates 0·68, 0·67, 0·57, 0·65 and 0·61μ per minute (28·5°).

(*f*) Two hyphae measured; growth from 170 to 2700μ; rates 1·37 and 1·43μ per minute (24·5°).

(*g*) Five hyphae measured; growth from 200 to 4200μ; rates 0·81, 1·06, 0·89, 1·01, 1·13μ per minute (26°).

(*h*) Five hyphae measured; growth from 400 to 1000μ; rates 1·44, 0·58, 0·67, 1·42, 1·53μ per minute (22·5°).

It will be seen that on the whole there is a fair consistency in the rates so measured, though here and there discrepancies occur, *e.g.* in the last two examples given.

Naturally the mean rates of the different experiments vary with the temperatures used. The method used is not a good one for determining the influence of temperature on the rate of growth, because a large number of experiments is required at each temperature to average out the individual variations, and the labour becomes very great. But the results over a range of 10° are given in Table II. At all the temperatures stated (except 21°–22°, where only one experiment was made) the figures are the mean of 6–10 experiments. Alongside the times at each temperature is placed in brackets a figure which expresses the ratio between the time required at that temperature and the time required at 22°. The agreement in the bracketed figures for any one temperature shows that the curve of total growth is similar at all the temperatures examined with the possible exception of the highest, viz. 28°–29°. The times required at 21°–22° are less than those at higher and lower temperatures, indicating that the optimum lies between 18° and 23°.

TABLE II

Temperature	Time in mins. required for growth from				Temp. coefficient for 1° C.
	300—600 μ	600—900 μ	900—1200 μ	300—1200 μ	
18°–19°	333 (1·54)	253 (1·54)	214 (1·39)	800	1·086
21°–22°	216 (1)	162 (1)	153 (1)	531	
22°–23°	228·7 (1·06)	184 (1·13)	162·3 (1·06)	575	0·9836
25°	243·8 (1·13)	180 (1·11)	175·4 (1·14)	599·2	
28°–29°	488·8 (2·26)	445·4 (2·75)	421 (2·75)	1355·2	0·7920

In the extension of the fungal hypha we seemed to have found a very striking and very simple case, in which the true rate of growth could be determined directly from the increments alone, without the necessity of taking into account any concomitant increase in the substance growing. Since extension occurs only at the tip and the other parts of the hypha take no share in the process, the growing area is small and approximately constant in size, and the true rate of growth is given directly and simply by the rate at which this point advances. Such a simplification would be of great value in the study of the much debated question of the reason for the early increase in growth rate.

It was, however, not quite certain that the already formed parts of the hypha do not contribute to the growth of the tip, although not themselves taking part in the extension. It was conceivable that the segments behind the tip took in food material and passed it along, either before or after elaborating it further, to the tip, where the final building was done. This would mean that this material passed through the septa on its way to the tip, and the septa in *Botrytis*, though sometimes incomplete, are usually complete partitions, ingrowths from the walls of the hypha. It does not follow that they are impermeable even when complete, but the only direct evidence obtained on the point indicated that they were impermeable to many substances. When the hypha is immersed in a weak solution of a dye, such as eosin, for example, the segments stain very unequally. One will take up the dye (and it clearly enters the interior of the segment) freely, staining deeply, while the adjoining segment may remain almost or entirely unaffected. In these circumstances the stain never passes through the septum from the stained to the unstained segment. The septa were impermeable to the dyes tried. But they might still, of course, be permeable to the nutrient or other materials of the fungal protoplasm. It is difficult to devise a method of testing this directly. Amputation of the tip of the hypha, though practicable, seemed so drastic a proceeding as to give no very good basis from which to draw conclusions.

One can, however, obtain some evidence in an indirect way. *Botrytis* in course of time sends out aerial hyphae, conidiophores, which rise vertically into the air. These hyphae presumably derive their food from the nutrient materials at their base, which must therefore pass along the length of the hyphae. Such hyphae also grow only at the tip, are also septate and in stains behave like the ordinary submerged or terrestrial hyphae. It would seem then that

in the case of aerial hyphae the food must be passing through the septa, and that this is therefore possible in the case of the other hyphae also.

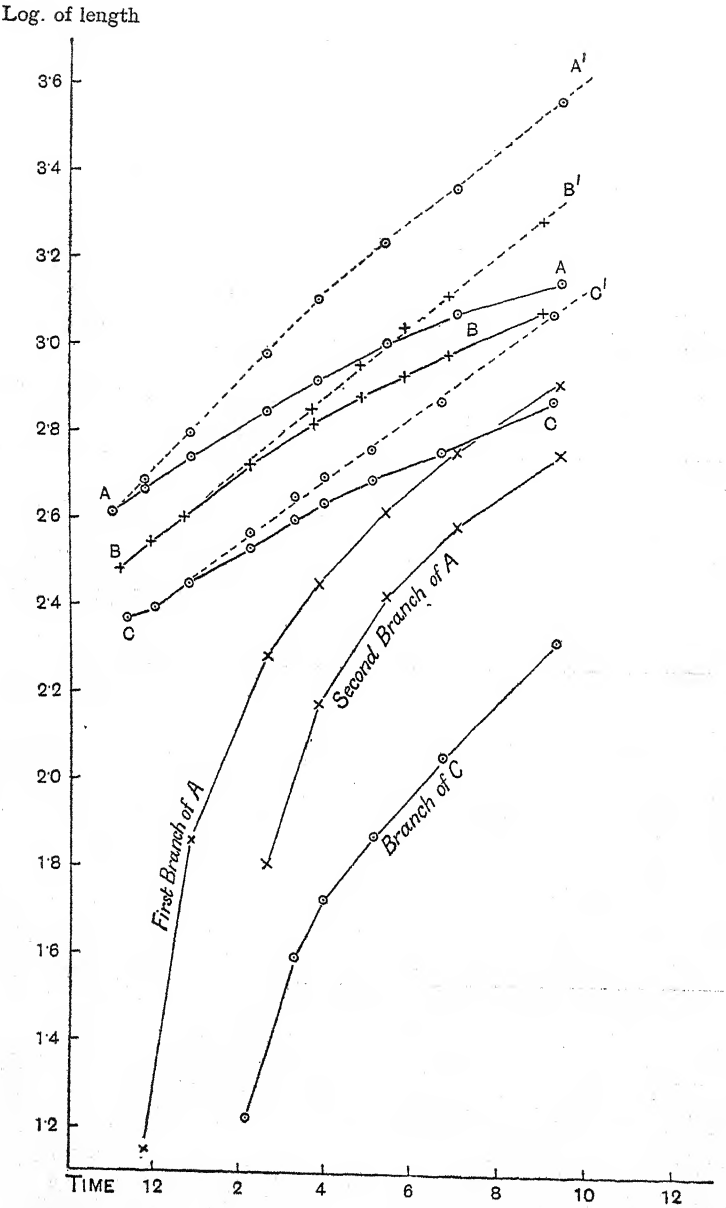
There are, further, some facts which indicate that such transference does actually occur. Absolutely, as we have seen, the rate of growth of the tip is rising continuously; but relatively, when compared to the length of the hypha, the rate of growth is continuously falling. This is most easily seen by plotting the logarithms of the lengths at various times against these times. If the amount of growth made, *i.e.* the increments of length, were constantly proportional to the total length of the hypha, increasing as it increased, then the resulting graph should be a straight line. In Graph II (see also Table III) there are plotted against time the logarithms of the

TABLE III

Lengths in μ at different times of (*a*) the main hypha alone, (*b*) the main hypha + all branches, for each of three hyphae *A*, *B*, *C*; and in the case of *A* the lengths of the first two branches. *A* was grown at 22°, *B* at 25° and *C* at 28·5°

A						B			C		
Time h.	m.	*a*	*b*	First branch	Second branch	Time h.	m.	*a*	*b*	Time h.	m.	*a*	*b*
11	2	410	410	—	—	11	11	304	304	11	20	233	233
11	47	469	484	14	—	11	55	350	350	11	58	246	246
12	52	549	622	72	—	12	41	404	404	12	49	283	283
2	39	700	955	191	64	2	15	526	526	2	15	341	367
3	53	825	1254	279	150	3	44	656	706	3	19	393	442
5	28	1002	1683	410	264	4	51	754	896	3	59	431	494
7	5	1180	2258	569	381	5	53	841	1089	5	6	486	570
9	28	1380	3556	802	552	6	51	944	1280	6	44	564	734
						9	3	1172	1901	9	16	729	1162

lengths of three hyphae *AA*, *BB*, *CC*, which are given as examples of what is an almost invariable occurrence. It will be seen that the graphs instead of being straight are curved, concave to the time axis. The relative growth rate is falling continuously throughout. This is very marked in the case of branches. Three branches are shown in the figure, branches from two of the main hyphae shown, and it is evident that their growth rate is very large at first, but falls quickly and would eventually approximate to that of the hyphae from which they are derived.



Graph II. Logarithms of the lengths of three hyphae (*A*, *B* and *C*) plotted against time: also of three branches of these; also of the sums of the lengths of each parent and all its branches and sub-branches (*A*—*A'*, *B*—*B'*, *C*—*C'*).

If, however, we plot against time the logarithms, not of the parent hypha alone, but of the sum of the lengths of the parent and all its branches and sub-branches, then we do get an approximately straight line (see Graph II, *AA'*, *BB'*, *CC'*). In other words, when we consider the developing fungus as a whole unit or single plant, then the total increment is constantly proportional to the total length, and the relative growth rate is approximately constant.

This was found to be the case in the great majority (70 per cent.) of all cases examined, during the period over which examination was practicable. It is not possible to continue for more than 10 or 12 hours measuring with any accuracy all the branches and sub-branches. They become eventually too numerous, and tend to dip into the agar and leave the optical plane of measurement. It cannot therefore be said how long this relationship would continue to hold good, and there are indications that the relative rate begins eventually to fall off. But during the period when such measures are practicable, it is the general rule that the relative growth rate remains the same or nearly so. It certainly does not increase.

It is difficult to account for this result, unless we assume that the rate of extension, *i.e.* the growth of the tip, is affected in some way by the parts of the hypha lying further back; but on that assumption we may suppose that what occurs is something of this kind. All along the hypha food is being taken in through the walls and passed along, either before or after further elaboration, to the tip, the mechanism of the transference being in the main diffusion. As the hypha increases in length, the total amount of food so taken in increases, because of the increase in absorbing surface. While the hypha is still very short, all the food taken in will be almost immediately available for use by the tip, which will respond to the increasing supply of food by increasing activity, and therefore by increasing rate of growth. During this period, which will usually be very short, the growth rate of the tip will correspond to the length of absorbing hypha. If the newly-formed parts of the hypha reach at once their full absorbing or at least full elaborating capacity, the growth of the tip will correspond to the length of hypha, and the increment of length be proportional to the total length of the hypha; if not, the relation between total length and increment will be altered, but the tip rate will still increase. As the hypha becomes longer, however, and septa are laid down, translocation from the oldest portions of the hypha, *i.e.* those most distant from the tip, will become more and more difficult and more slow. This will cause

an increasing concentration of the food in these areas, and it will also have its effect on the tip. The concentration throughout the hypha will no longer be uniform, but a pressure gradient will be formed, rising from the tip backwards. The concentration at the tip will be less than the mean concentration of the whole length of hypha. The tip, therefore, while it still continues to increase in speed, will no longer advance at a rate proportional to the whole length of the hypha, but will fall more and more behind that rate. The curve will assume the shape shown by the parent hyphae in Graph II.

If now a new point of extension, a branch, arise from the zone of increased concentration, the conditions are restored nearly to those prevailing when the parent hypha was short and unbranched. The new point avails itself of the food as it comes in near its point of origin, and once more the total increment will bear some relation to the total length of the hypha. The process will go on indefinitely, but the relative growth rate will gradually decline.

It is not suggested that this sketch of what is occurring in the hypha is anything but the merest outline, incapable of being filled in with detail owing to the existence of many other incalculable factors which come into operation. But, crude as it is, it gives a first approach to an explanation of the facts observed; the gradual rise of the tip rate to a maximum, the gradual decline of the increment of length as compared with the total length in the parent hypha, and the approach to a constant rate when the sum of the increments of length is compared with the total sum of the lengths of parent and branches. It offers an explanation of the origin of branches (which arise when the concentration in a segment reaches a high value), and of the high rate of their growth when they first appear (since they arise from a zone of concentration already high).

Whether, however, this sketch does or does not correspond at all to what is actually taking place, it does seem to be most probable that the portions of hypha behind the tip are contributing in some way and to some extent to the activity of the tip. The absolute rate of growth of the tip, therefore, does not by itself give a true measure of the real rate of growth. To determine this accurately would require an estimate of the amount of the contribution and the quantity of substance contributing, and this we have no means of making. But from what has been already said it is clear that there is nothing to suggest that any increase in true rate is occurring, such as is found in the case of bacteria. When the fungus is considered as a whole,

the amount of growth at any time is very nearly proportional to the length (and therefore to the mass) of the fungus, with a tendency to diminish rather than increase; and when the individual hypha is considered by itself, apart from its branches, the rate of growth relatively to the length falls continuously.

It has frequently been suggested that growing organisms produce some substance which accelerates their own growth, and numerous attempts have been made to express the curves of growth by formulae based on such a supposition. There has not as yet been found in this enquiry into the growth of *Botrytis* any evidence which makes such a hypothesis necessary. The facts so far established are explicable on other and possibly simpler lines, and if such acceleration were actually occurring, one would expect to find some indication of it in the experiments described.

Summary

The fungal hypha elongates at the tip only. In *Botrytis* the rate of elongation, at first very slow, steadily increases as time passes, reaching eventually a maximum value (probably determined by some limiting factor) at which it remains constant for a time. Considerable differences in the actual rate and in the manner of development occur in different individual hyphae, but under similar conditions of environment the large majority behave similarly.

Although the actual extension occurs only at the tip, there are grounds for believing that the parts of the hypha behind the tip contribute to the activity of the latter. The rate of extension, however, of the individual hypha, taken by itself, is not constantly proportional to the length, or mass, of the hypha, but, relatively to the length, falls off continuously as the hypha elongates.

The branches also show an increasing rate of apical elongation, and in each branch, taken by itself, the extension relatively to the length falls off continuously as the branch elongates. But if the whole hyphal system, *i.e.* the parent hypha with all its branches and sub-branches, be taken as one unit or plant, then the rate of growth of the whole system remains approximately constant for hours. A tentative explanation of these rate relationships, the origin of branches, etc., is offered.

No evidence has been found of any increase in true growth rate, such as has been found in bacteria, nor anything which suggests the formation during the hyphal development of any substance accelerating its growth.

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THE FACTORS GOVERNING BUD FORMATION: A CHAPTER OF PLANT PHYSIOLOGY

(Continued from p. 49)

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CONTENTS

CHAPTER VI

SEASONAL VARIATIONS IN THE FOOD RESERVES OF THE SHOOT. THE CARBOHYDRATE : NITROGEN RATIO

IT has already been suggested that the question of the localisation of food reserves needs considerable study before the relation of these reserves to bud growth can become at all clear.

While this particular field can be regarded as unexplored as yet, attempts have been made recently to work out precisely the nutrient relations in the tree at different seasons of the year and to determine the significance of these in the processes which result in bud formation. Investigations of this type have been carried out chiefly in American research stations and fall roughly into two classes.

(1) Determinations of the carbohydrate, nitrogen and ash constituents of the various parts of the tree at the end of, or during, the well-marked growth periods of the year, *e.g.* during the rest-period, at leaf-fall or at the time of bud-break.

(2) The establishing of a connection between the nutrient relations of the plant, as modified in various ways by external treatment, and the response made by the plant in the direction of sexual reproduction.

Of the first class the memoir of Butler, Smith and Curry (1917), on the physiology of the apple, records an attempt to investigate the variations in the plant body of the reserve food materials during the seasons of growth and quiescence. The periods of vegetation chosen were dormancy, swelling of the buds, time of blooming, cessation of active growth and leaf-fall.

The parts of the tree examined were (1) the current season's growth, (2) the one-, two-, three-, four-, and five-year old branches, (3) the large and small roots and (4) the trunk.

As the data were based on the analyses of two trees only the authors did not consider their figures absolute on account of the magnitude of the individuality effect. The figures for the remaining parts of the tree, therefore, are expressed relative to those of the trunk as unity, the trunk being assumed to be that portion in which metabolic activity is the least marked.

Their results may be summarised as follows:

(1) The storage carbohydrates are starch and saccharose. During dormancy the roots contain relatively more starch than the trunk and the branches less. At bud-opening the starch in the branches increases, several peculiarities of distribution being present. The most marked increase is in the three-year old branches. When the trees are in bloom the roots and the one- and four-year old branches contain relatively more than the trunk, but at the close of the period of active growth the trunk contains relatively more than either roots or branches with the exception of the three-year old branches.

Generally, for all parts, the starch curve shows a maximum at the point when the buds are swelling and a secondary maximum when the leaves are falling or a little before. Minima occur at dormancy and when the tree is in bloom.

The distribution of the saccharose is more markedly affected by the state of vegetation of the tree. During the dormant period all parts contain much more than the trunk, varying from one and a half times in the five-year old branches to six times in the small roots.

These latter have lost heavily by the time the buds open, while the one-year old branches have gained. There are no great changes in the remaining parts. At flowering the small roots are again relatively richest and remain so for the rest of the season.

As the changes in saccharose-content were largely independent of the changes in the starch, the authors concluded that saccharose is primarily a reserve food material not simply a stage in starch condensation. The accumulation of saccharose is sometimes coincident with starch storage however, *e.g.* in the small roots and in the two- and three-year old branches.

At the awakening of vegetation, hydrolysis of starch is not very marked except in the two- and three-year old branches, the supply of carbohydrate material for the growing parts being mainly furnished by the saccharose stored in the young roots.

(2) The reducing sugars present an interesting type of distribution. In the small roots there is always a greater abundance of these than in any other part, especially during dormancy, and at the time of bud-swelling when there is a relatively enormous increase. There is a corresponding but smaller increase in the large roots.

On comparing the data for these sugars with those obtained for saccharose the authors drew the conclusion that, at the beginning of the season, reducing sugars are formed at the expense of saccharose the hydrolysis being most marked in the small roots. It proceeds actively in the one-year old branches but an increase of reducing sugar is not apparent here as it is used up in the manufacture of new tissues. Evidently the reducing sugars of the small roots are translocated to the growing parts prior to the opening of the flowers and it is only after flowering that the tree becomes independent of its carbohydrate reserves.

The authors have no doubt that saccharose is the chief translocation form of the carbohydrates.

(3) The nitrogenous reserves have a somewhat different distribution for they are mainly stored in the young roots and one-year old branches. From both these there is a gradual decline during the period of active growth but after the cessation of growth the quantity increases to a maximum at the time of leaf-fall. It was concluded, therefore, that the nitrogenous reserves are translocated from the young branches to the actively growing regions upon the awakening of vegetation.

(4) The fat-content was represented by the ether extract and a liberal allowance must be made for the probable impurity of this.

The largest variation in the fats was found in the young roots and in the one-year old branches. From the time of dormancy until the time of blooming the former lost gradually while the latter made a corresponding gain. The quantities in the larger roots were smaller and behaved only approximately like those in the smaller roots. From flowering-time to the end of active growth there was a rapid increase of fats in the small roots and an almost corresponding decrease in the one-year old branches. The older branches showed an approximately similar behaviour but there were minor fluctuations as the age of the branch increased. The explanation of the authors was that the fats acted as reserve materials, more especially in the roots, from which they were translocated at the awakening of vegetation to the branches where they were in part re-formed.

(5) Ash. From dormancy until the awakening of vegetation the one-year old branches became relatively richer in ash but poorer from this point onwards. In the case of the older branches these fluctuations were not so great. Correspondingly, the ash in the young roots first increased and then decreased from the time of budding until the close of the season. Both branches and roots were always relatively richer in ash than the trunk. The large roots showed only small fluctuations until the tree bloomed when there was a steady gain until active growth ceased.

The sole conclusion drawn by the authors was that there is a slight passage upwards of ash constituents from the small roots into the one- and two-year old branches.

(6) Phosphorus and potassium. The distribution of these shows that probably the phosphorus required at the awakening of vegetation is obtained from the younger branches. Very little evidence of the translocation of potassium appears until the tree blooms when there is a passage upwards from the older branches, *i.e.* from the parts which are older than those which supply the necessary phosphorus and nitrogen.

After making all necessary reservations the above may be regarded as a valuable contribution to our knowledge of the subject. The authors suggest a different scheme of carbohydrate economy from that generally accepted and certain of their introduced variations are not easy of acceptance, *e.g.* the independence of the starch and saccharose contents. In other cases the explanation is far from satisfactory as in the account of the translocation of the nitrogenous reserves on the awakening of vegetation.

Nevertheless a picture is given of the character and relative

quantities of the food reserves available at the end of the dormant season for utilisation by the buds, developed during the previous growing season, in their first spring burst of respiratory activity and to serve as a foundation for future development.

Although Butler, Smith and Curry regarded saccharose as the principal translocation form of the carbohydrates their evidence for this is not convincing. According to their data the saccharose of the young roots forms the chief source of the large increase of reducing sugar at the time of bud-swelling and the relation between the starch, saccharose and reducing sugars of any particular portion of the tree at a given period remains obscure. What is really needed is a more intimate knowledge of the relation of individual buds to the materials actually available locally and this was beyond the scope of the research under discussion.

Another investigation of this type was that of Mitra (1921) who studied the subject in fruit spurs and in seedling apples. He made frequent periodical determinations of starch, sucrose, maltose and glucose during the growing season and also determined the changes of acidity in autumn, winter and spring. His results for the sugars agreed fairly closely with those of Butler, Smith and Curry with the exception that he asserted a maximum for the starch of the roots and one- to two-year old apple stems in October and November, with a secondary maximum in June, in both cases slightly later than they were fixed by the first-named authors. Mitra also showed a correlation between the acidity of the tissues and the activity of diastase and maltase, but the figures he gives show such slight fluctuations that the existence of the correlation cannot be regarded as established.

We may turn now to the investigations of the second class which have led to the proposal of a most suggestive theory of the relation between fruit production and the nutrient conditions in the plant.

As a result of a biochemical examination of the tomato plant, Kraus and Kraybill (1918) have developed the theory that the behaviour of the plant, as regards vegetative growth and sexual reproduction, depends to a large extent upon the relative proportions of the available carbohydrates and nitrogen in the plant body. They recognised the following four classes into which a plant might fall as regards these proportions.

I. Carbodydrates deficient and limiting, moisture and mineral nutrients very abundant: Vegetation will be weakened and the reproductive function restricted.

II. Carbohydrates increased above the limiting value, nitrogen and mineral nutrients as above: The plant will become vigorously vegetative as a result but coupled with this will be barrenness and sterility.

III. Carbohydrates very abundant, nitrogen inadequate: Vegetative development will be lessened but the conditions will be favourable for sexual reproduction on account of the accumulated carbohydrates.

IV. Carbohydrates very abundant, nitrogen still further reduced and now the limiting factor: Vegetative growth and sexual reproduction will both be suppressed.

In other words, fruitfulness is associated with a condition of balance between the carbohydrates and nitrogen. Several interesting biochemical relations were established by modifying the external factors—soil, water and nutrition. For example, increased nitrogen, and more especially the nitrate-nitrogen, was associated with a decrease of pre-reducing substances, sucrose, polysaccharides and total dry matter.

Again, under conditions of abundant available nitrogen the withholding of moisture increased fruitfulness in the same way as limiting the available nitrogen supply.

Kraus and Kraybill also demonstrated for the complete plant a descending gradient of total nitrogen and moisture in the stem from the top to the bottom. The gradient for total dry matter, sucrose and polysaccharides was, on the other hand, ascending.

Emphasis was laid upon the necessity for a complete knowledge of the specific environment of the plant and also of its different parts, in order to account for the great variations in amount of carbohydrates in different parts of the same plant and in plants grown in different conditions of nutrition. In dealing with cultural practices they enunciated the following guiding principles.

I. Parts of stems or cuttings containing a large amount of carbohydrates produce roots abundantly when supplied with moisture.

II. Fertilizers containing available nitrogen mainly produce vegetative response but may at the same time increase or decrease fruitfulness according to the available carbohydrate supply.

III. Moisture supply increases growth or fruitfulness only when accompanied by an available nitrogen supply. Both are made increasingly available by cultivation of the soil. If both are already present, decrease of cultivation will encourage fruitfulness.

The following modifying tendencies of certain cultural operations were also deduced.

I. Pruning promotes or retards fruitfulness by balancing the carbohydrate supply in the plant with the available moisture and nitrogen supply.

II. Ringing is also effective by modification of the carbohydrate:nitrogen relationship.

In conclusion Kraus and Kraybill made it quite clear that not only environmental but also hereditary factors must be considered in attempting to explain the reproductive and vegetative behaviour of plants; for both functions are closely associated, being not only concerned with the slightly modified vegetative parts but also with the highly specialised portions known as fruits. The degree of modification in the latter is dependent upon physiological changes within the plant and may vary greatly in the same variety and even in the same individual.

In a record of work carried out at the Oregon Agricultural Experiment Station during 1918–1920 the Director gives a summary of the results of an experimental search for suitable indicators of the internal condition of the tree. Leaf tissue was chosen on account of the ease with which this could be handled in chemical analysis, and three different treatments of fruit trees were employed in the investigation, viz.:

(1) Heavy application of sodium nitrate in April;
(2) Heavy root-pruning in March—no nitrate;
(3) Controls.

By June 5th the leaves of the nitrate-treated trees were very dark green, those of the root-pruned smaller and yellowish and those of the controls intermediate. These differences were more pronounced on July 26th when collections of leaves were made. Chemical analysis of the leaves showed the following C:N ratio.

Treatment	C:N ratio
Heavy nitrate	6·03
Root pruned	9·98
Control	8·48

From these figures it was claimed that the leaves are probably very good indices of the general condition of the trees, but no mention is made of the relative performance of the three classes, the connection between fruit-bud formation and a high C:N ratio being assumed.

In the same report a summary is given of pruning investigations on tomatoes, the object being to find a method of modifying the C:N ratio. The results showed that:

(1) Unlike the apple the tomato spur cannot be affected, as far as number of blossoms and amount of set are concerned, by the foliage in close proximity to the spur but is probably dependent for its carbohydrate supply upon the plant as a whole.

(2) Both pruning and nitrate supply determined the amount of growth, blossoming and set of fruit for the plant as a whole. Root pruning had but a slight effect, if any.

(3) Chemical analysis showed a strict correlation between the amount of pruning and the percentage of total carbohydrates in the plant, but a similar correlation could not be established for the total nitrogen. All pruned plants, however, contained a higher percentage of nitrogen than the unpruned. As pruning increased in amount the C:N ratio diminished. Root pruning rather increased the amount of nitrogen.

Further studies have shown that the tomato will only set fruit when there is a fair amount of foliage on the plant. The removal of leaves almost automatically checks setting.

In an investigation of the nutritional changes in fruit-spurs Harvey (1921) studied the effect of defoliation on the formation of fruit buds, the guiding principle being the work of Kraus and Kraybill which had not previously been applied to perennial plants. He re-calculated certain of the data of Smith, Butler and Curry (1917) and obtained the following seasonal averages for the C:N ratio.

	C	N	C:N
Dormancy	52	24	2·1
Buds swelling	71	28	2·5
In bloom	41	20	2·0
Active growth ceased	62	17	3·6
Leaf-fall	61	21	2·8

The specific aim was to determine the extent to which the leaves of the apple control its metabolism and the precise significance of the C:N ratio.

This was done by defoliation of the fruit-spurs of ten trees each of three varieties, half the spurs being defoliated and the other half de-fruited and used as controls. The material was sampled three times during the season, viz. immediately after and two and five weeks after defoliation. In each sample a division was made, "new growth," *i.e.* wood of the present year, being separated from "old growth," *i.e.* the growth made in previous seasons.

The effect of defoliation upon the production of fruit-buds is shown below and it will be noticed that there is a pronounced varietal difference present.

Name of variety	Percentage of fruit buds		Percentage performance of defoliated spurs
	Control	Defoliated	
Jonathan	16	6·2	38·7
Grimes	13	7·1	54·6
Wagner	18·1	10·6	58·6

The chemical data are presented under three headings.

I. *Moisture and Insoluble Solids.*

(*a*) The moisture content of both defoliated and control spurs diminished steadily during the experimental period.

(*b*) On each date of sampling

(i) the defoliated spurs showed a higher moisture content than the controls;

(ii) the "new growth" of a spur showed a higher moisture content than the "old growth."

(*c*) A varietal difference was demonstrated, the Wagner spurs having the greatest moisture content.

II. *Nitrogen.*

Owing to their time-consuming nature nitrogen determinations were only made on the samples of July 5th.

(*a*) There was a greater amount of nitrate-nitrogen in the defoliated spurs especially in the "new growth."

(*b*) Soluble nitrogen—less nitrate-nitrogen—decreased in the "new growth" but fluctuated in the "old growth." More was found in the defoliated spurs than in the controls.

(*c*) Protein-nitrogen: The defoliated spurs contained more than the controls.

III. *Carbohydrates.*

(*a*) *Reducing sugars.* The new growth always contained greater percentages than "old growth" and defoliated spurs than the control. The total in all spurs diminished steadily during the experimental period.

(*b*) *Sucrose.* This showed considerable variation and no conclusion could be drawn from the figures.

(*c*) *Hydrolyzable polysaccharides.* These did not increase during the experimental period. The controls showed a maximum on July 5th but not the defoliated spurs the normal processes of which had probably been retarded. The controls contained more than the defoliated spurs.

Harvey remarked on the fact that the pentosans and lower pentose sugars were not specifically determined, although these are

present in apple tissue in sufficient quantity to be of considerable importance in view of the probable general vital function in metabolism of the pentosans and monosaccharide pentoses to the water-holding capacity of the cells.

The results of all the chemical analyses undertaken were summarised by saying that the defoliated spurs contain more than the controls of water, reducing sugars, total sugars and total nitrogen but less soluble and insoluble solids and hydrolyzable polysaccharides. Also the C:N ratio was greater in the controls than in the defoliated spurs.

Harvey advanced a theory of the restriction of fruit-buds based upon the applicability of the C:N ratio. In the interpretation of a response the carbohydrates are assumed to be the factor limiting fruit-bud formation; if these are decreased, the nitrogen remaining undisturbed, the conditions no longer allow of this. He thought that it should be possible to select a group of spurs of the non-productive Class IV and, by defoliation, cause a greater production of fruit-buds and change them to Class III.

Gourley (1915) showed that nitrate formation in the soil to the extent of 20–40 parts per million was essential for maximum vigour of fruit trees and abundant fruit-bud formation. An excess above this proportion would not give any increase of either. He also found that the largest number of fruit-buds was produced when the moisture ran the lowest during the period of fruit-bud formation and where good growing conditions were present earlier in the season. No relationship could be traced between the rainfall of the growing season and fruit-bud formation.

Very many more works have been published on the general nutrient condition of fruit trees and its modification by seasonal and other external factors; but as they make no further contribution towards an analysis of the relation between this condition and fruit-bud formation they need not be considered. An attempt to get closer to the problem was however made by Hooker and Bradford (1921) who, in a study of the relation between growth and performance, came to the conclusion that the C:N relationship was in no way causal nor did it represent a relationship of fundamental importance to the plant.

Their studies were carried out on very large numbers of apple spurs of several varieties. They found a strong tendency towards a continuing increase of fruit-bud formation with increase of growth and consideration of the data by years led to the conclusion that the

condition of the whole tree influences spur performance. An association was indicated between the condition of the tree and the character of the distribution curve of bud formation with respect to length of spur.

Analysis of the data by trees showed a tendency towards mass behaviour on the part of the spurs independent of the growth of the individual spur, while analysis by branches showed for annual bearing trees a pronounced individuality of behaviour.

The authors deduced the existence of a general influence affecting fruit-bud formation from the behaviour of non-bearing spurs of biennial-bearing trees. An increase or decrease of vegetative growth almost invariably occurred inversely with the crop. They also considered it probable that influences farther back in the tree than the spur, *e.g.* in the branch or scaffold limb, were decisive in promoting fruit-bud formation. If these influences were strong they caused general uniformity, but when less positive the performance of the individual spur might be decided largely by the conditions within the spur itself.

Certain considerations were advanced as the result of chemical analyses. It was found that the bark of the limbs of a tree in full bearing contained as high percentages of potassium as the spurs but only half the nitrogen. But, while the nitrogen content of the bark was similar in bearing and non-bearing trees, that of the spurs was much greater in the bearing trees. The percentage of starch was less in both in the bearing years and the authors thought that this was an indication that much of the aerial portion of the tree acted as a unit. They were of the opinion that, although the starch content of the bark might not affect that of the spurs, there was a probability that the circumstance preventing starch accumulation in the latter might prevent the same thing in the bark when the majority of the spurs were bearing fruit. Further, it might be supposed that the large amount of developing fruit would utilise all the carbohydrates that the diminished leaf area of the bearing tree can manufacture and consequently decrease the supply reaching the storage regions.

The authors saw a connection between this and the results obtained by Hartig (1858) who found in the beech and oak that, previous to the seed year, large amounts of starch were stored in the medullary rays of the wood. In the seed-bearing year the starch content of the entire wood was reduced to a minimum, the accumulations of eight years being consumed, while, in addition, nearly all the nitrogen disappeared from both wood and bark. They considered that Hartig's results were important as indicating that, in the beech and oak, as

well as in the alternate bearing apple, the tree acts more or less as a unit and that not only might the starch content of the wood be significant but that a direct relation was probable between the reserve starch of the trunk and the fruitful condition of the tree as a whole.

They suggested that if Hartig was correct in viewing the employment of the starch accumulations in the trunk for seed production, the question of the passage of carbohydrates up the trunk ought to be studied from a new point of view, especially when it is considered that, in the beech for example, no significant upward translocation of carbohydrates would occur eight years out of nine.

The most important conclusion of Hooker and Bradford was that their evidence indicated that, although conditions within the spur are important and frequently decisive, conditions in the tree behind the spur are equally important and as frequently decisive of the performance of the spur itself. Consequently the factors which determine fruit-bud formation must be localised narrowly sometimes and widely at others.

In spite of the objection of these authors, sufficient evidence appears to exist to justify the conclusion that the magnitude of the C:N ratio represents a real causal relation between the nutrient reserves of a tree and the intensity of fruit production although it must be admitted that the precise significance of the water relations in this connection has yet to be determined. The importance of the ratio is not detracted from by the fact that opposing views are held as to whether the fruitful condition is associated with the starch or nitrogen content nor are these views necessarily divergent.

Much of the value of this work arises from the fact that additional significance is given to the basis and results of such operations as defoliation, pruning and the application of fertilizers, but it must be emphasised that only the general nutritive condition of the tree has been investigated and much additional work of the type of that of Magness and Jesenko, to be described in the following chapter, is needed before an improvement of our position in this respect can be hoped for.

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CHAPTER VII

MISCELLANEOUS EXPERIMENTS BEARING UPON THE PROBLEM OF FRUIT-BUD FORMATION

Scattered throughout the literature are a large number of memoirs describing investigations not necessarily directed primarily to the problem under discussion but from which a quantity of useful evidence bearing upon it may be extracted. In addition to these there are a number of researches, dealing directly with the problem, which cannot easily be classified under the headings of the preceding chapters.

Edminster (1917) made a statistical study of some nine thousand apple shoots in an attempt to elucidate the relation between the angle, length and diameter of shoots and the production of fruit spurs from lateral buds. He concluded that while there is doubtless a correlation between angle of shoot and the percentage of buds breaking, on the average this percentage is greater in the case of the more upright than in that of the more horizontal or drooping shoots. The percentage of lateral buds which form fruit-spurs is also higher.

A comparatively high degree of correlation was found to exist between the length of shoots and the percentage of lateral buds which formed fruit-spurs. The stouter shoots also were more productive of both side shoots and spurs.

A positive correlation was also demonstrated between the three factors—angle, length and diameter, but each factor was more or less independent of the others.

Magness (1917) carried out defoliation studies on dwarf six-year-old apple trees in which the following types of defoliation were practised.

(1) Bulk defoliation—in which all the leaves from large portions of the trees were removed.

(2) Defoliation as pruned—where all the leaves were removed from the same places and to the same extent as in summer pruning.

(3) Removal of all the leaves from certain of the current season's shoots.

(4) Defoliation of individual spurs.

(5) Removal of the leaves from individual axillary buds. The results of the studies were summarised by Magness as follows:

(*a*) Fruit-bud initiation will not take place and fruit-buds will not form in the absence of a fair amount of leaf area in the tree; but leaf area in one part of the tree will not usually supply food material to the buds of another part to the extent necessary to cause them to produce fruit-buds. Defoliating one-half of a tree has little influence upon the undefoliated portion, but that which is defoliated functions as it would if all the leaves were removed from the whole tree.

(*b*) Food material stored in the tree during the dormant season is largely stored in the tissues adjacent to the leaves in which it was manufactured. This is borne out by the fact that, during the following spring, the defoliated portion does not develop so strongly as the undefoliated portion.

(*c*) Removing the same number of leaves as in pruning, without any pruning, has the same effect upon fruit-bud formation for the year following that a summer pruning would have which removes leaves from the same portion to the same extent. Buds on one-year wood which has been defoliated are slower in starting into growth the following spring and make a weaker growth than do other buds on similar but undefoliated shoots.

(*d*) A shoot is largely dependent upon its own leaves for nourishment. In one variety the axillary bud seemed to be entirely dependent upon its subtending leaf for its carbohydrate supply.

(*e*) Removal of the subtending leaf entirely prevents fruit-bud formation and buds so treated either remain dormant during the following season or form very weak shoots.

(*f*) There is a decided increase in the number of crystals of calcium oxalate in the tissues of defoliated buds which may be indicative of a small supply of carbohydrates and general slow metabolism in the bud tissue.

The investigation of the factors governing bud formation commenced at Long Ashton by Barker and Lees (1916) is still being prosecuted and, in connection with this, the present author has carried out intensive defoliation experiments upon shoots of the apple during the period of active extension growth.

In one case two pot-trees of Brayton Hall and Woolf River were employed and, beginning on June 6th, 1921, by which time the

terminal buds of last year's shoots had commenced to grow out, the leaves of these were amputated in order as soon as the young petioles were long enough to cut. The result was the formation of a deformed and contracted club-shaped shoot. The defoliated shoot made very little growth but its diameter became greater than normal. The lateral buds remained minute and resembled the three dormant buds usually to be found at the base of a normal shoot. In this condition the shoot was allowed to winter.

When the shoot was only partially defoliated the internodes of the undefoliated portion reached the normal length while in the defoliated portion the internodes were contracted and the minute axillary buds crowded on the swollen shoot.

In the Spring of 1922 the only buds to break were the terminal and those of the undefoliated portions. In every case the terminal continued to elongate almost normally but none of the minute defoliated buds could be induced to grow out even when the terminal bud was amputated.

In other cases, in addition to the shoot produced by the terminal, the shoots which arose from the lateral buds were also defoliated. In 1922 the new terminals gave rise to flower shoots but no growth could be obtained from the tiny laterals of the, now, dwarfed shoot. Further back on the parent shoot, the defoliated products of last year's laterals gave no growth at all, while other untouched laterals which had gone back in infancy the previous season now made strong shoot or spur growth. Even the two or three buds above the usual dormant group at the base now gave rise to small shoots with two or three leaves each.

Results such as the above could only be explained on the assumption of a "Loeb Effect" if this were further assumed to be restricted in operation to the shoots of the current year.

A more extensive series of defoliation studies was carried out upon trees representing a cross between the two varieties Kingston Black and Medaille d'Or. Each experimental shoot was divided into three regions, a basal division containing the six lowest buds and the internodes below them, a mid-region comprising the next eight to ten buds and internodes above these and an apical region formed by the remaining portion.

Owing to the fact that defoliation was not commenced until June 6th, the leaves of the two lower regions had almost reached their maximum size before the operation whereas defoliation of the apical region was carried out as the leaves expanded.

The Spring of 1922 was characterised by an unusually strong tendency to break on the part of the lower lateral buds, even in those varieties which generally produce much bare wood. Control shoots invariably showed a good break from the apex down to about five or six buds from the base.

Defoliation of the basal region led to one or two buds remaining dormant in addition to the usual three. In no case did one of the basal three grow out and invariably the two lowest subtending leaves were found on defoliation to be much less than normal in size.

Defoliation of the mid-region, after the leaves had attained their full size, exercised a small but definite check on the breaking of the lateral buds. Moreover, the shoots which developed from these buds in the following season were, in degree of development, far behind similarly situated shoots on the controls.

So far, the correlation between development of the axillary bud and persistence of its subtending leaf appears to be real inasmuch as the longer the leaf is allowed to remain the greater is the progress made by the bud. Complications were encountered, however, in the case of the apical portion where the terminal bud and (sometimes) two or three immediately below this gave slight indications only of being affected adversely by defoliation, although the length of the internodes was diminished as before. To the end of the period of extension growth this extreme apical region is constantly surrounded by a group of young leaves and in this respect differs from any other portion of the shoot. In all probability the progressive development of the terminal of a defoliated shoot is to be connected with this.

None but quantitative modifications were observed and it is obvious that the factors decisive qualitatively must be sought for much earlier. This demands further study of the normal annual periodicity of both leaf and flower buds and the determination of normal shoot succession over a period of years. Only in this way is it possible to decide whether, by appropriate manipulations or modifications of the external factors, a desired qualitative response in the direction of bud development is obtainable or not.

Studies of this kind have been commenced by Blaauw (1920) and his pupils, Luyten (1921) and Versluys (1921), who have studied the periodicity phenomena of bud development in the plum and cherry respectively. In the plum the vegetative-point of the future flower-bud can be recognised thirteen months before differentiation of the floral parts begins.

In another type of investigation Gardner (1917) studied the effect of bending artificially one-year-old apple shoots. He found the percentage of buds breaking on the bent shoots to be practically the same as that on the controls except in one variety where the bending acted as a stimulus rather than as a check. Generally bending gave rise to a change in the location and distribution of fruit spurs and new shoots upon the shoots of the preceding season, its tendency being to increase the number of fruit spurs towards the terminal end and to decrease them towards the base. On the other hand, its tendency was to decrease the leaf shoot growth from the terminal portion and increase it from the base. No indication was given of a physiological isolation of the portion below the bend as Loeb conjectured to be the case in bent shoots of *Bryophyllum*.

Attempts have often been made to modify the rest-period of the buds of woody plants generally in the direction of an abbreviation. Some of the methods employed, *e.g.* etherisation and the treatment of the aerial portion in the warm water bath, have been developed to the point of practical economic application. Some of these attempts are of interest as throwing light upon the problem under discussion.

Jesenko (1912 (i)) sought to abbreviate the rest-period by plunging woody twigs bodily into a bath containing alcohol or one of various acids. The cut end was kept out of the bath.

Working with pear twigs cut on November 30th he found that these, after being bathed with 5 per cent. ethyl alcohol for twelve hours at a temperature of 14° C., showed signs of bud break on December 12th, eight days before the controls and six days before twigs which had been similarly treated with water. Higher concentrations had not such a favourable action. If the treatment were delayed until January the water bath gave but a slight positive effect while the alcohol bath was decidedly harmful.

Similar results were obtained with hydrochloric acid in strengths of 0·5 per cent. to 5 per cent.

In the case of the plane, hornbeam and elder the response to the chemical bath was irregular while *Salix aurita* yielded consistently negative results.

In general Jesenko claimed to have demonstrated that the bursting of the buds of woody twigs could be accelerated by the employment of baths, during the resting period, of hydrochloric, sulphuric and malic acids and also of tap-water and water saturated with carbon dioxide. The higher concentrations he found to work most satisfactorily about the middle of this period while, towards

the end, very dilute solutions were necessary. After emerging from the rest-period the buds could not be further stimulated by treatment with any of the above.

The same author also studied the effect of hypodermic injections and simple punctures upon the buds of woody shoots which had been defoliated so late in the season as to preclude the possibility of the axillary buds breaking during the same season as would have been the case had the operation been carried out earlier. Two small trees of *Tilia grandifolia* were defoliated on July 24th, one being employed as a control and the other for the injection experiments (1912 (iii)).

The buds of the latter were injected with alcohol in strengths of 1·5 or 10 per cent., ether in strengths of 0·1 and 1 per cent. or plain water. The results are summarised below:

Fluid employed	Result of injection
Alcohol, 1 %	Buds began to swell 7 days after injection
Water or 1 % Ether	" " " 8 " " "
Alcohol, 1 %	" " " 12 " " "
Ether or Alcohol 10 %	Buds remained closed
Controls	Only a few buds deep in the crown of the tree developed

After beginning to swell the buds continued their progress, those injected with water being the best. Similarly treated buds on twigs of which the leaves had been allowed to remain showed no sign of growing out and Jesenko thought that the subtending leaf inhibited in some way the growth of these buds which would otherwise have broken in consequence of injection.

In experiments with *Fagus sylvatica* Jesenko found it necessary to take precautions so that the puncture or injection was made in such a manner as not to injure the growing-point. Moreover, care had to be taken not to inject into the woody cylinder in which case no effect followed.

Buds also developed into good shoots after being cut slightly in the middle or after the tip had been cut away. Buds which had not been injured in this manner did not grow out.

Jesenko obtained negative results with *Quercus*, *Sorbus* and *Carpinus*. These he ascribed to the smallness of the buds which led to their suffering extreme injury when punctured. With the larger buds of *Acer* and *Syringa* positive results were always obtained.

The most interesting relation established by Jesenko was that between the strength of the fluid required for the stimulation of bud-growth and the appropriate season for its employment. In spring

the concentration required became gradually smaller while in autumn the reverse was the case.

The difficulty in interpreting the results of experiments of this type is to divorce the wound reaction from the chemical stimulus both of which act in the same direction. Experiments carried out by the present author (1922) with apples showed that fairly large punctures near the base of presumably dormant buds would cause a small quantity of growth while annular cortical rings round the base often stimulated these to increased lateral shoot growth. The effect, however, which was roughly proportional to the initial size of the bud, was neither great enough nor sufficiently regular to show that the wound stimulus in itself was intense enough to do more than initiate growth, while microscopic examination failed to show that the starchy food reserves were more abundant in one part of the shoot than another.

It was thought, however, that there might be a possibility of the local store of carbohydrates not being immediately available to the young growing-point owing, for example, to the appropriate enzyme being deficient or absent. It was decided therefore to see whether the former might not be made increasingly available by the injection of a suitable enzyme like diastase. The effect of the fluids generally employed in injection experiments has been uniformly a lowering of the surface tension of the protoplasm with a corresponding diminution of its permeability. By injecting diastase it was hoped to act solely on the carbohydrate food reserves. Some wound reaction was naturally to be expected and this was controlled by injections with distilled water in the case of other buds.

The most striking result was given by a last year's shoot of a Brayton Hall apple. This had 34 buds and, at the time of injection, Nos. 1 to 28 had just begun to break. Nos. 29 to 34 showed no signs of breaking, although the upper three were decidedly larger than Nos. 32 to 34.

Three buds were chosen for injection, viz. Nos. 18, 26 and 31. Needle punctures were made on each side of the bud, extending below the base of the growing-point, and into these an injection of 5 per cent. commercial diastase was made with a hypodermic syringe. On the second day after injection an appreciable effect was to be remarked in Nos. 18 and 31, while, by the sixth day, all three had gone ahead No. 18 having outstripped the terminal. The buds were re-inoculated on the sixth and sixteenth days after the start but, while Nos. 18 and 31 continued to outstrip the rest, No. 26 ceased to

make further progress from the sixth day. No. 31 continued to elongate and, by the end of the growing season, had produced a thin lateral shoot over 20 cm. in length.

In the Spring of 1922 the terminal bud of this shoot grew out strongly, but the buds below were less than normal in size and the leaves they produced less than the average in area. Bud No. 18 produced a similar but slightly smaller shoot. A further sign of subnormality was proved by the fact that these two shoots alone were attacked by mildew (*Podosphaera leucotricha*): there was no trace of this fungus on the rest of the tree. Barker and Lees (1919, p. 96) found that the shoots which arose below the ring in response to May ringing had long internodes, were of small diameter and often contracted mildew.

The remaining non-injected buds, with the exception of the five at the base which remained dormant, gave rise to small leaf spurs with three to seven leaves each.

In the second year (1922) the remaining buds again produced leaf spurs with the exception of the terminal and No. 3, which gave rise to fruit spurs, and Nos. 29, 30, 32, 33 and 34 which made no growth at all.

It is clear, therefore, that the injection of diastase may cause a lateral bud to make more growth than it otherwise would have done, but this growth is subnormal as the progress in the second year and the susceptibility to mildew proved. Further, the available store of soluble carbohydrates was evidently insufficient to produce a normal vigorous shoot but in the absence of further knowledge of the quantity of development innate in an individual bud, it is unprofitable to speculate as to the possibility of supplying sufficient carbohydrates to any of the lower buds to produce a normal vigorous shoot.

In the Spring of 1921 additional information was gained as a result of injecting apple twigs with various organic and inorganic solutions. Curtis (1918) had already published the results of his work upon the stimulation of root growth in woody cuttings by treatment with various chemicals. In most cases the twigs appear to have been immersed for a certain time in a bath of the solution and, after being washed, grown on either in distilled water or in moist sand. The chemical mostly employed was potassium permanganate, but solutions of other manganese salts were also worked with, in addition to salts of iron, calcium, boron and other inorganic substances. In the second part of the investigation Curtis studied the effect of treat-

ment with solutions of organic substances, amongst which were saccharose, glucose, asparagin and peptone. His attention was primarily directed to root formation but certain effects upon the top growth of the cuttings were recorded and these are of interest here.

He found that when unripe succulent twigs of *Ligustrum*, taken on August 30th, were brought into distilled water after having been allowed to stand for three days in solutions of cane sugar of 1, 5 and 10 per cent. respectively, the resulting root growth was markedly increased. The greatest growth was shown by the cuttings which had been treated with the strongest solutions. Not only was there an absorption of the sugar but this was converted into starch and stored in the cutting in that form. If immature twigs were allowed to stand in the sugar solution for at least two days, sufficient food was stored to permit of growth of the tops as well as of the roots. Fully ripened cuttings left in 5 per cent. cane sugar for fourteen days showed a very marked swelling of the basal 3–4 cm. which was absent from the controls.

The cuttings died eventually if left continuously in sugar solutions, but mature twigs, brought into distilled water after treatment for two to fifteen days with sugar solutions of from 0·2 *M* to 0·4 *M* concentration, showed increased top growth of a somewhat peculiar character. In this the maximum development of shoots came from the 2nd–4th nodes from the top and was coupled with partial or complete inhibition of growth of the buds of the uppermost node. This occurred even when the lower buds were darkened and the uppermost buds exposed to the light.

Curtis advanced two alternative explanations of this disturbance of the normal order of development. On the one hand he considered that the sugar might have increased the concentration and osmotic pressure in the cells of the lower part of the twig and thus have given rise to a reduction of the water-content of the upper portion; or an increased supply of organic food material might have been made available for the lower buds which would in consequence grow out earlier and more rapidly than the upper buds. He stated that, in practically all cases, the upper portion became withered and shrunken apparently supporting the first explanation but that this withering did not become evident until some time after the new shoots were formed. The present writer (1922) has found in similar experiments that the exact point in time at which this shrinkage takes place is difficult to determine owing to the retention of its rigidity by the cortex for some time after the shrinkage really begins.

Curtis also forcibly injected cuttings of tomato and privet with sugar solutions and greatly increased their sugar-content. He found a distinct retardation of bud development in *Ligustrum* irrespective of the nature of the injected fluid. Cuttings which had been under the suction pump started growth about forty days after being set out while the untreated shoots started in ten days.

The Long Ashton experiments (1922) were carried out with apple cuttings taken just before the commencement of the growing season of 1921. The sugars employed were saccharose, dextrose, and levulose in 0·5 per cent. solution while other shoots were injected with tap-water and 5 per cent. diastase respectively.

The cuttings were as uniform in size as possible and were injected in a container which was exhausted by means of a suction pump. After rinsing they were grown on in tap-water.

Preliminary experiments were performed with a 1 per cent. solution of watery eosin to gain some idea of the distance to which a watery solution would penetrate under the conditions of experiment. In the central wood cylinder it was possible to trace the stain with diminishing clearness for a distance of 20 cm., but the penetration to the individual buds did not correspond to this regular gradient for buds were at times completely missed, the fluid failing to penetrate the vascular strand. It is obvious, therefore, that if the action of the injected fluid is strongly localised the possibility of irregular penetration becomes important.

The injected shoots showed no retardation of bud break as recorded by Curtis for *Ligustrum*. The three buds at the apex were later in breaking than those of the middle and basal regions and this relative rate of progress was maintained throughout.

The cuttings injected with water were more advanced than the controls, while, for the first twenty-three days, the former were about as good as the saccharose injected shoots. During the next nine days, however, the buds of the saccharose cuttings went ahead of the controls. Those injected with dextrose and levulose behaved similarly, but diastase was comparatively inefficient. This was doubtless to be connected with irregular penetration owing to the nature of the injected solution.

A parallel series was set up in which the cuttings after injection were ringed to a width of 10 mm. in the fourth internode from the base.

The result was the same in every case; the three buds below the ring grew out but before any growth could be made by those above the

portion of the shoot there became desiccated and shrunken. Evidently the loss by evaporation from the ring was sufficiently great to prevent the water lost by the upper portion from being made good by the ascending current. That this was most probably the case was demonstrated by the author by potometer experiments to be described elsewhere.

The following conclusions can be drawn from the above results when combined with those of Curtis.

(1) Bud growth can be accelerated and increased by the injection of twigs with solutions of sugars and, perhaps, diastase.

(2) Irregularities of bud break, *i.e.* the so-called disturbances of polarity, may be due wholly or in part to irregularities of penetration by the injected fluid.

(3) Bud break and development are contingent upon the water-content being maintained. The osmotic concentration alone is insufficient to do this in the face of severe competition for water such as that set up when there is strong evaporation from the surface of a ring.

Later on it will be shown that, if the buds are in full growth at the time of ringing, they are able to maintain a continuous supply of water to the portion of the shoot above the ring. Before passing to a consideration of experiments upon this point mention might be made of a discussion by Curtis (1920) of the possibility of a stimulatory action upon bud development of increased aeration consequent upon mechanical injury of the tissues near the bud when the shoot is ringed. He considered that Loeb had overlooked this point in explaining the development of shoots from the buds on the side of a shoot from which the leaf had been removed. Curtis stated that, after the rest-period has been passed, any bud of a twig which has been coated with paraffin can be induced to develop, while the others remain dormant, by cutting the paraffin coat in such a manner as to allow of its aeration. Especially is this the case if an opening is made to the leaf-scar when there can be no question of a mechanical weakening of the paraffin layer.

The following experiment performed by the present writer shows that this can, at most, be a subsidiary factor.

A paint was prepared by adding powdered animal charcoal to a gelatine solution until on drying this gave an opaque horny substance. The buds of apple shoots were then coated with this paint shortly before bud-break was expected. In one case a shoot possessed twenty-three buds all of which were coated, with the exception of

the basal six which were very small. During the first ten days no change was observed but on the next day the coat of the terminal bud cracked. Four days later the fourth bud from the top burst its coat and, like the terminal, began to develop into a vigorous shoot. On the twenty-fifth day the first two uncoated buds had protruded a green tip but beyond this they made no progress and went back to a desiccated condition.

On the thirtieth day the coating was removed from the upper fifteen buds which appeared green and healthy. They did not grow out, however, and rapidly became desiccated in spite of favourable external conditions. The result was that the shoot was bare except for the shoots produced by the terminal and fourth bud.

In the second year these two continued to produce extension shoots while the remaining laterals as far as No. 19 produced leaf-spurs of five to nine leaves each. Nos. 20 and 21 gave rise to very small spurs with two or three rudimentary leaves each while the two extreme basals did nothing at all again.

Experiments of this class always show clearly the reality of the polarity of the normal shoot as a definite phenomenon. The terminal has always the greatest potentiality of bud-break while that of the rest decreases generally basipetally, if not always quite regularly. As the same thing is repeated in the extension growth shoot which arises from the terminal during the following season it appears probable that this gradient of growth potentiality is not only laid down in the growing-point but is virtually independent of the local food reserves.

Once more therefore the importance is emphasised of a precise knowledge of the normal development phenomena of the bud before attempting to interpret what may be merely phenomena of periodicity as effects of modifications of the external or internal factors. Most of the observations described in the foregoing chapters have been carried out during one growing season upon buds which were already fully differentiated. Investigations of the behaviour of buds during the winter or resting period have been few and disconnected but certain of these throw some light upon the conditions which influence bud development and are dealt with separately in the following chapter.

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IMPERIAL BOTANICAL CONFERENCE

THIS conference will be held at the Imperial College of Science and Technology, South Kensington, from July 7–16, 1924. The subscription for attending the Conference is one pound, and those intending to be present are requested to notify the Secretary (Mr F. T. Brooks, 31 Tenison Avenue, Cambridge) as soon as possible.

The Preliminary Programme, of which copies can be obtained from the Secretary, includes papers and discussions on the following topics: The best means of promoting a complete Botanical Survey of the Empire, Correlation of Taxonomic Work in the Dominions and Colonies with Work at home, Vegetation Survey in different parts of the Empire, and Training in Field Ecology, The Economic Possibilities of Plant Breeding, The Value of Selection Work in the Improvement of Crop Plants, Cold Storage of Apples, Crop Physiology—modern methods of attack, Obscure Plant Diseases of Widespread Occurrence, The Relation of Plant Pathology to Genetics, and of Forest Pathology to Silviculture, Education and Research, and Miscellaneous. Excursions to Wisley, Merton, Blakeney Point, Rothamsted, Kew, and Cambridge will take place in the following week.

THE CYTOLOGY OF *MATTHIOLA INCANA* WITH REFERENCE TO THE GENETICS OF CERTAIN CULTIVATED VARIETIES

By IRENE M. ALLEN
(Late Bathurst Student, Newnham College, Cambridge)

(With forty-four figures in the text)

I. Introduction

The long series of results obtained by Miss Saunders from breeding experiments on *Matthiola incana*, carried on over a large number of years, and the recent papers (20–24) published on the cytology of both plants and animals, particularly the work of Morgan and his collaborators in America on *Drosophila ampelophila*, suggested that an enquiry into the cytology of *Matthiola* (18) might be of interest.

Matthiola produces constantly two types of singles—the "no-*d*-singles" which breed true, and produce pure singles only, and the ever-sporting or double-throwing singles ("*d*-singles") which give both singles and doubles. The genetical facts are explicable on the supposition that at least two factors, *XY*, are concerned in the production of singleness, doubleness being due to the "absence" of either or both factors. In the pure-breeding single the factors *X* and *Y* appear to be linked—in the *d*-single they are not. The probability is that in the *d*-single the pollen is all of one kind, and that it lacks the factor for singleness, but the ovules—from the evidence afforded by reciprocal crosses—are of two kinds, single-producing and double-producing. The Sulphur White variety which has also been investigated is peculiar in that it is ever-sporting as regards both plastid colour and doubleness. Doubleness, therefore, although apparently unrelated to sap colour or surface character is linked with the factor for plastid colour. This investigation, however, deals mainly with the relation of singleness and doubleness to the cytological structure.

II. Methods

The flower buds were fixed with 1 per cent. chrom-acetic solution, with weak and half strong Flemming's solution, and with acetic alcohol mixtures. The best results were invariably obtained with the acetic alcohol mixtures.

III. PRE-MEIOTIC MITOSES

The pre-meiotic mitoses in *Matthiola* have been studied in the root tips of germinating seedlings, and in the archesporial divisions of ovules and anthers for comparison with meiosis.

In the archesporial divisions, the nucleus, in the early stages, presents a coarsely granular appearance (Fig. 1), in great contrast to the "resting stage" following the telophase of the last archesporial division in the anther (Fig. 9)—to be described later. This difference is probably due to the fact that the archesporial mitoses follow each other in quick succession, there being no real condition of rest. The granules are not isolated, but are connected by a delicate reticulum. Typically a single large nucleolus is present, though the condition with two equal sized nucleoli is more frequent than in the meiotic prophase and seems to be unconnected with degeneration. No trace of nucleolar budding has been observed at this stage.

It is difficult to decide whether a single continuous spireme or a number of separate threads is eventually formed, but in whole nuclei—*i.e.* nuclei in which the nuclear membrane is seen to be intact by careful focussing—free ends are apparently found, so that the evidence is in favour of the latter supposition. In any case the chromatin granules tend to become aggregated at certain points, and a superficial examination would suggest that at this stage a large number of isolated segments are present. On closer examination many of these are seen to be connected by delicate fibrils, and it is probable that the chromatin is at first unevenly distributed on the ground substance of the chromosome, and that later the distribution becomes more uniform (Figs. 2–5).

Ultimately the somatic number (14) of the chromosomes may be recognised, at first as elongated thickened rods, intertwined or wound spirally around the periphery of the nucleus. Later they thicken still further, and finally become bent, so that from now onwards, in polar views of the equatorial plate, and in figures of the chromosomes upon the spindle they appear as U- or V-shaped structures. No trace of a split has been observed in the chromosomes during prophase, but each V-shaped chromosome splits longitudinally on the equatorial plate, and the daughter chromosomes proceed to the poles. There is apparently no trace of any precocious longitudinal splitting during anaphase or telophase (Figs. 6–8).

As is frequently the case, the elongated rod-like form of the chromosomes of the pre-meiotic mitoses differs very considerably

from the constricted dumb-bell shape of the heterotype and homotype chromosomes (cf. Gates, *Oenothera*(7); Blackburn and Harrison, *Rosa*(2)), but there is no apparent morphological dissimilarity between the chromosomes.

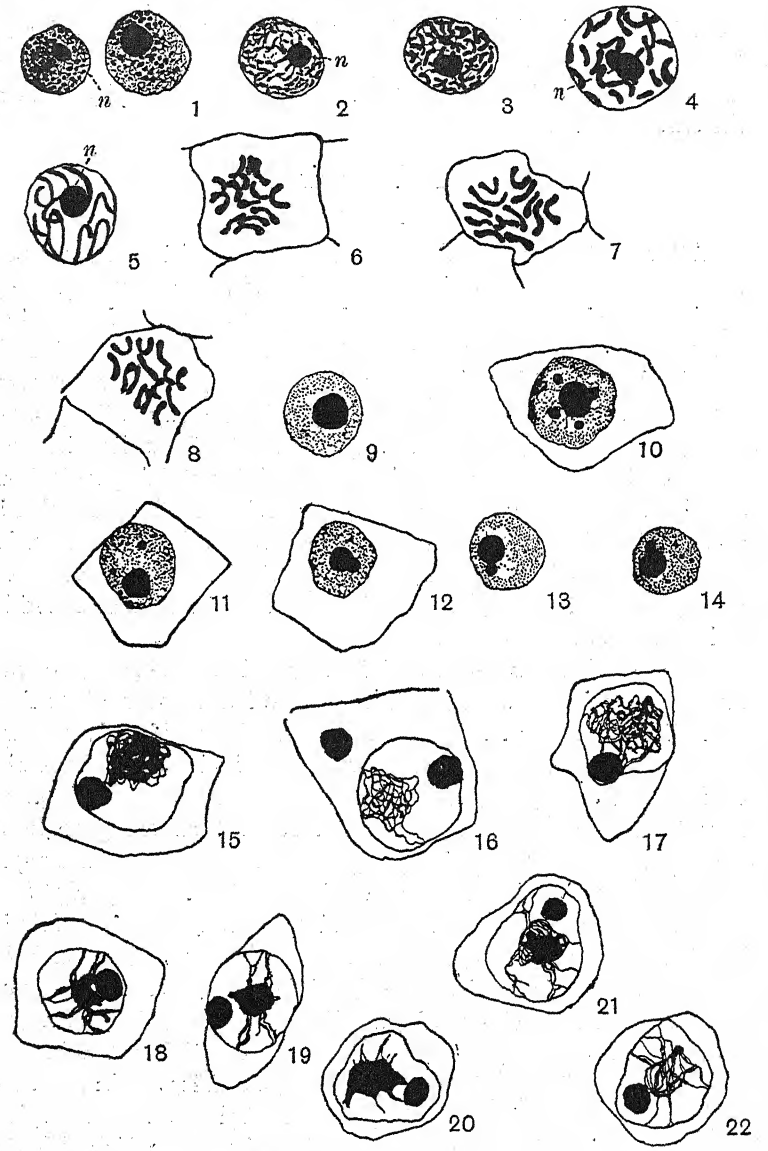
POLLEN FORMATION

IV. Meiosis

After the telophase of the last archesporial division in the young anther the nucleus passes into a condition of complete rest (Fig. 9). Typically a single large nucleolus is present, but occasionally two rather smaller and apparently equal-sized nucleoli are present, such nuclei being almost invariably on the edge of the loculus, where they degenerate. The nucleolus is surrounded by a comparatively clear space, traversed only by a few very delicate fibrils, whilst the periphery of the nucleus is occupied by a faintly staining reticulum, showing no trace of chromatin aggregation. The resting condition is somewhat prolonged, for in a number of preparations all the nuclei in the anther are in this stage, whereas in later stages, which are more rapidly passed through, an almost complete series is to be found in a single loculus.

The first sign of renewed activity is seen in the behaviour of the nucleolus, which now proceeds to bud off a number of smaller nucleoli (Figs. 10–14). This process of nucleolar budding is unusual among the higher plants, and unlike the discharge of chromatin bodies described by Miss Digby(5) the nucleolar buds in *Matthiola* do not pass beyond the nuclear cavity. Nucleolar budding has been described by Miss Curtis in *Synchytrium endobioticum*, the fungus responsible for "Wart Disease" of the potato(3). In *Synchytrium* the chromatin component of the bud is dissolved, whilst the linin is distributed throughout the nucleus. The reticulum of the *Matthiola* nucleus becomes increasingly chromatinic, and shows a number of fine, but rather deeply-staining fibrils which are finally connected up to form a delicate, much convoluted chromatinic network. The nucleolar buds disappear during the process, and it would seem that they are concerned in the supply of chromatin to the developing spireme. During these stages, the archesporial cells, which hitherto formed a compact mass, first begin to show signs of separation.

The leptotene stage just described is followed by the stage known as zygonema, in which the pairing of whole chromosomes is believed to take place. The staining capacity of the threads has increased to a slight extent, but they are still of an extremely delicate character,



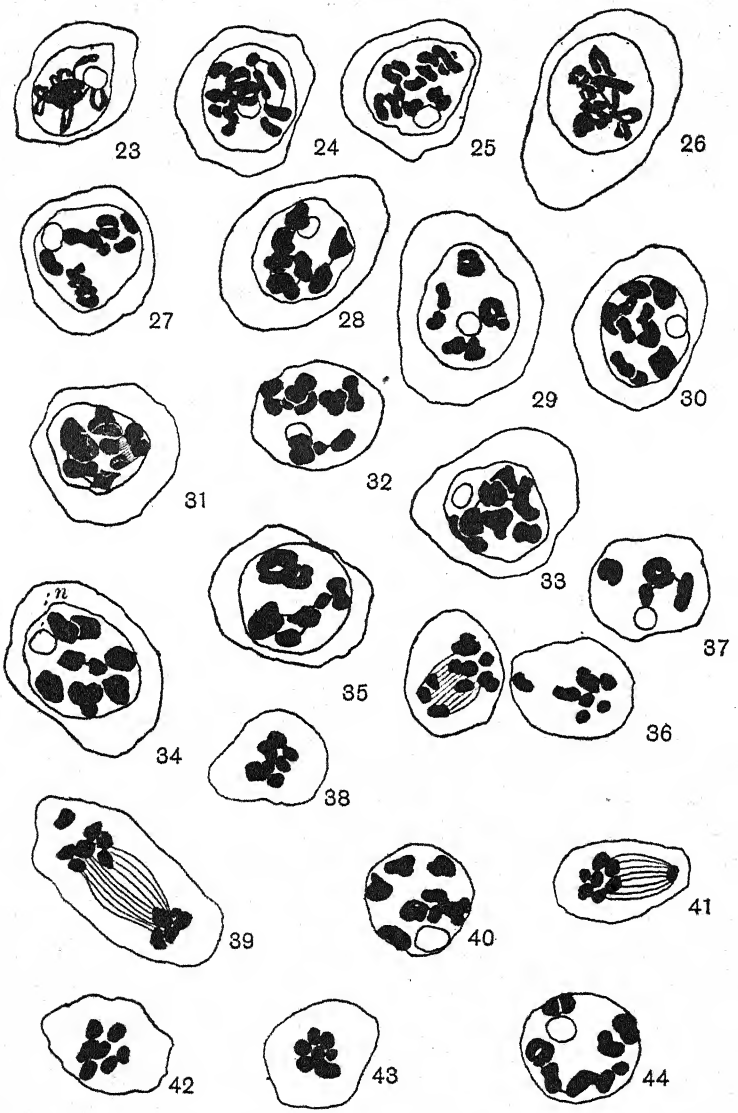
Premeiotic Mitoses. Figs. 1–8

(Drawn from slides of a pure red single variety)

Figs. 1–5. Stages in the formation of the somatic chromosomes. Figs. 6–8. Polar views of the equatorial plate showing the fourteen chromosomes.

Meiosis. Figs. 9–44

Fig. 9. Resting nucleus after the last archesporial division. Figs. 10–14. Nuclei in early stages of meiosis showing nucleolar budding. Figs 15–17. First contraction figure. Figs. 18–22. Second contraction figure.



Figs. 23 and 26. Further stages in the formation of the heterotype chromosomes.

Figs. 24, 25. Temporary dissociation of univalents.

Figs. 27–44. Heterotype mitosis, showing the number (seven) and ring form of the bivalent chromosomes, and the characteristic links.

(Figs. 28–38. Ever-sporting single. Figs. 39 and 42. Pure red single. Figs. 40, 41, 43, 44. Sulphur white varieties.)

and it is well-nigh impossible to say whether the duality indicated is a result of side-to-side pairing of homologous chromosomes (parasyndesis), or whether it is due, as the adherents of telosyndesis would affirm, to the precocious appearance of the longitudinal split in preparation for the ensuing mitosis—in the substance of a single chromosome. In the light of subsequent events the parasyndetic view is maintained.

Zygonema is followed by the first contraction figure (cf. Farmer and Moore (6)). A single large nucleolus is characteristic of this, and of succeeding stages. The tightly coiled mass of chromatinic threads is usually to be seen towards the periphery of the nucleus on the side remote from the nucleolus, but the latter may occasionally become entangled in the contracted mass, or may be connected with it by one or two threads (Figs. 15–17).

The first contraction figure is also a protracted stage, but sooner or later the knot begins to unravel. The chromatin threads have contracted rather considerably, and when unravelled appear a good deal shorter and thicker, and consequently take the stain more deeply. All evidence of duality is temporarily obscured. The process of contraction continues, and typically a stage is found in which a number of chromatin threads (approximately equal to the haploid number of the chromosomes) radiate from the remains of the knot as centre. The position of the nucleolus at this stage is variable. The duality in the threads again becomes obvious, and is much more definite than in the zygotene stage.

The second contraction figure follows; and during this contraction the shortening and thickening of the filaments progresses still further, so that when the knot unravels, the heterotype chromosomes can soon be defined. At this point the bivalent chromosomes appear as split rods—homologous chromosomes being closely associated, and elongated and rod-like in form. Contraction proceeds until each univalent member of the pair is dumb-bell shaped, usually exhibiting a well-marked transverse constriction. Next there is a temporary dissociation of univalents (Figs. 24–25), an unusual occurrence in plants, and I have found no record of it elsewhere. It is, however, well marked in *Matthiola*, and the figures bear a striking resemblance to Agar's figures of the spermatogenesis of *Lepidosiren paradoxa* (1). The reunion (presumably of chromosomes which were originally paired) is soon effected. As there is no morphological distinction between individual chromosomes, one can only infer that the repairing is between homologous chromosomes, but from present day

knowledge of the behaviour of the chromosomes this inference can be justified (Figs. 18–26).

The typical form of the heterotype chromosomes of *Matthiola* is that of a closed ring. This form is arrived at in several ways. Generally the two homologous univalents become loosely attached by a thread of linin, and the ring form is attained by one of the univalents swinging round on this as a hinge. Occasionally, however, the dumb-bell shaped univalent becomes drawn out in the region of its constriction, and pairs with its fellow, and an elongated, dumb-bell shaped bivalent is formed. This bends round on itself in the thinned-out region to form the ring (Figs. 27–37).

About this time the nucleolus disappears, but if it should persist longer than usual it need cause no confusion in counting the chromosomes, as it stains much more faintly with iron-alum-haematoxylin. In many cases (Figs. 34, etc.) the bivalent chromosomes show connecting threads or bands at this stage. These connecting links stain deeply with iron-alum-haematoxylin, as if composed of chromatin. The occurrence of these connecting links has apparently not been previously reported. The only references I have found which might apply to something of a similar nature is again to be found in Agar's paper on *Lepidosiren*. Agar(1), however, regards the connecting links between the chromosomes as composed of linin, brought into prominence by overstaining, and merely serving to orientate the chromosomes in the heterotype spindle by means of their elasticity. Sections of *Matthiola* which are, if anything, understained, have been examined from this point of view, and the links still appear to stain as chromatin.

The haploid number of the chromosomes in *Matthiola* (estimated from counts of the bivalents, and from polar views of the equatorial plate in heterotype and homotype mitoses) is *seven* (Figs. 41, 42, etc.). The number in the pure single strains is identical with that in the ever-sporting or double-throwing strains investigated. (The figures are mainly drawn from preparations of a pure-breeding glabrous red variety, but a complete series of stages has been worked through, both for the pure single, and the ever-sporting single. The Sulphur Whites have also been investigated, and the cytology of all these forms is apparently identical.) Thus the differences between the *d*-single and the no-*d*-single varieties are not due to any variation in the chromosome number. The heterotype division is the reductional division, the rings breaking apart on the heterotype spindle, and the half-rings (whole univalent chromosomes) are always accurately

paired, and there is no evidence of any irregularity in distribution such as is described so frequently among *Oenothera* hybrids (Gates (7–10), Davis (4)), where two or more homologous chromosomes may pass to the same pole, whilst the other pole receives no representative of this chromosome pair (Figs. 37–43).

The chromosomes pass to their respective poles, and the nuclei are reconstituted. The longitudinal split, which is to take effect in the homotype mitosis, and which frequently appears precociously in the whole chromosomes on the heterotype spindle in anaphase or telophase, cannot be seen in *Matthiola*. The homotype mitosis follows immediately upon the heterotype, and though the nuclei are reconstituted, the chromosomes never really lose their individuality. Their outlines may become ragged, but the seven chromatin blocks are always seen. The homotype division is equational, the dumb-bell shaped univalents of the heterotype splitting longitudinally and half-chromosomes passing to either pole. The homotype daughter chromosomes still retain the constricted dumb-bell shaped form seen in the heterotype division, but after the homotype division the chromosomes of the daughter nuclei become alveolated and break up, and the nucleus assumes a finely reticulate condition.

The majority of plant cytologists uphold telosyndesis rather than parasyndesis (cf. Farmer and Moore (6), Gates (7–10), Davis (4), Miss Digby, etc.), and Hogben (13), discussing the problem of synapsis suggests that far too much stress has been laid on the similarity of the meiotic phase in plants and animals. Whilst confirming Agar's scheme for various members of the Cynipidae, Ichneumonidae, and for *Periplaneta americana*, he compares his results with Miss Digby's recent work on the cytology of *Osmunda* (5), and suggests that the presence of *two* contraction figures in plants renders comparison difficult, if not almost impossible. The Second Contraction corresponds to the "Bouquet stage" and synizesis (synapsis) in *Lepidosiren*, but the first contraction has no parallel in Agar's scheme. *Matthiola* would seem to conform to Agar's scheme more closely than any plant hitherto described, but in common with the majority of plants it possesses two well-marked contraction figures. The pairing of the chromosomes, initiated in zygonema, is not completed in the first contraction, but the final and complete pairing of the chromosomes is deferred until the "Bouquet Stage" and second contraction. It is possible also that the significance of these contraction figures in the pairing of the chromosomes during meiosis has been largely over-estimated, and that the contractions are merely

the expression of some definite but purely physical condition of nuclear aggregation.

The development of the pollen of *Matthiola* shows, therefore, no apparent cytological difference between the pure single forms and the "double-throwing" singles. The embryo-sacs are now being examined from the same standpoint.

I wish to express my warmest thanks to Mr F. T. Brooks, under whose supervision these investigations have been made, for the advice he has given and the interest he has taken in their progress. I am also extremely grateful to Miss E. R. Saunders, at whose suggestion this enquiry was undertaken, for generously supplying me with material, and for her interest in the work.

V. Summary

1. The diploid number of the chromosomes in *Matthiola* is fourteen. There is no morphological dissimilarity between the chromosomes, and in pre-meiotic mitoses the chromosomes are U- or V-shaped.

2. A prolonged resting stage intervenes between the pre-meiotic mitoses and meiosis. A single large nucleolus is present. Nuclei in which more than one nucleolus is present appear to degenerate. Renewed activity is marked by a process of nucleolar budding—the "buds" supplying chromatin to the developing reticulum.

3. There are two well-marked contraction figures in *Matthiola*, the second contraction figure being followed by a temporary dissociation of univalents.

4. The typical form of the heterotype chromosome is that of a closed ring. The bivalents are frequently joined by connecting links or bonds, which apparently stain as chromatin. The chromosomes are always accurately paired, and there is no evidence of any irregularity in distribution during the reduction division.

5. The haploid number of the chromosomes in *Matthiola* is seven. The number in the pure single strains is identical with that in the ever-sporting or double-throwing forms investigated (including the Sulphur White). Thus the genetical differences do not appear to be attributable to any observable difference in chromosome behaviour during pollen formation.

The drawings were made with the aid of a camera lucida, and a 2 mm. Zeiss apochromatic lens with a number 12 ocular was employed throughout.

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THE FACTORS GOVERNING BUD FORMATION: A CHAPTER OF PLANT PHYSIOLOGY

(Concluded from p. 102)

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CONTENTS

CHAPTER VIII

THE RESTING PERIOD OF BUDS

IT has often been shown that an essential condition for vigorous shoot-growth in the Spring is the sojourn of the aerial portion of the plant in an atmosphere having an uniformly low temperature. Interesting studies of this type were made by Weber (1921), and Coville (1920) has published a popular account of experiments on the influence of cold upon the subsequent growth of *Vaccinium corymbosum* and other plants. The main thesis of the latter was that the trees and shrubs of cold climates become dormant at the end of their growing season without the necessity of exposure to cold, but that if they have not been subjected to chilling during this season of dormancy they will start into growth in the Spring much later than plants which have received this chilling. Relying upon data which showed that samples of dormant wood of *Vaccinium corymbosum*, taken in early Spring, possessed a ratio of sugar to starch which was seven times as great as that found in dormant wood taken in the autumn, he connected the effect due to chilling with the transformation of starch into sugars. He advanced the theory that before the period of cold the "amylotic enzyme" was unable to pass the "active cell membranes" and come into contact with the starch, and that chilling weakened the vitality of the membrane so that the enzyme was able to pass through it.

Coville gave instances also of dormant shoots which had been started into growth by other means than chilling. For example, a wild crab had remained dormant in a greenhouse during autumn and winter at a temperature of 55° to 70° F. On April 5th three branches were pruned and fifteen days later the uppermost bud in each case had begun to grow. On other unpruned shoots no bud growth had taken place. Two other wild crabs had received the same preliminary treatment as the above. On April 5th a ring of wood down to the bark was removed from one and fifteen days later the bud immediately below had begun to grow. On the second tree a bud immediately below a notch had begun similarly to grow out. Dormant buds of *Vaccinium* were caused to develop when the portion of the stem near them was rubbed hard with a knife handle.

Such reponses are of course commonly met with only they have generally been assumed to take place independently of any rest-period.

As the result of respiration studies Howard (1915) had concluded that the specific effect of all agents which break the resting period in woody plants was the stimulation of the enzymes to activity. He supposed the rest-period to begin with the inhibition of these by the products of their own activity during mid or late summer and that this inhibition was continued through the autumn when carbohydrates were present in excess. Towards the end of the rest-period the enzymes were assumed to become more active so that the beginnings of growth took place.

The physical properties of the cell membrane have been shown to be modified in the case of one agent responsible for the breaking of the rest-period, viz. wound reactions. Tröndle (1921) showed that the permeability can be depressed as the result of wounding to such an extent that the uptake of salts completely ceases. He also showed that the reaction decreased in intensity with the distance of the cell from the wound and with the strength of the wound stimulus.

Wiggans (1919) connected the resistance of buds to frost with a high sap concentration and showed that the later a fruit tree entered its rest-period the more prolonged was its dormancy and the less the likelihood of its buds suffering from frost.

Roberts (1917) found that in cherries which had suffered from frost all the injury was confined to the flower-buds. The trees which suffered least were later in blooming in the spring. The extent of injury decreased with the quantity of shoot growth, *i.e.* with lateness of maturity of the growth. Along the terminal growth the

susceptibility to injury was directly proportional to the degree of development of the lateral buds but those of the apex itself were less liable to injury than the laterals lower down the shoot. The terminal bud itself remained very free from injury, an additional instance of the reality of the polarity of the shoot.

Roberts held that size of leaf was a factor of prime importance in influencing the development of axillary buds. He also showed by defoliation experiments that the removal of the subtending leaf arrested the development of the accessory flower buds. Buds showing a minimum of development occurred on shoots which had been partly defoliated as the result of fungus attacks and also on very strongly growing shoots. Applying the theory of Kraus and Kraybill (Chapter VI) he claimed that in strongly growing young trees the carbohydrates might be used up in forming tissue and the flower buds be correspondingly arrested in development. More bud development would therefore be found in older trees which make less growth. He considered that carbohydrate formation would be below normal in partially defoliated trees with consequent less differentiation and that the same conditions might be characteristic of the base and tip of the growth shoots where less bud development was to be found in connection with the smaller leaves there.

In a second memoir Roberts (1922) published the results of a closer study of the seasonal development of the flower buds of the cherry and the relation of the stage of development to killing. He proved that the amount of killing was correlated with the degree of development of the buds after differentiation of the floral parts, and the hardiest buds were generally those which were least developed.

Fatal injury most commonly occurred in the pith of the pedicel and this was associated with a vacuolated condition of the cytoplasm of the cells of the susceptible area.

Roberts considered that this condition of the cytoplasm, or the physiological conditions attendant upon it, constituted the factor of susceptibility, for in hardy buds there was but little of this type of tissue. The principal interest from a practical point of view was held to be the question of how to reduce the amount of this cytoplasmic vacuolation which accompanies maturity of the cells. Together with the percentage of buds killed it was found to be reduced by appropriate cultural treatment, *e.g.* by pruning combined with the employment of sodium nitrate as a fertiliser, which appeared to afford a clue to a practical method of prevention.

The most important conclusion to be drawn from the work of

Roberts is that the effect of climatic factors upon bud performance must always be considered in relation to the stage of development of the buds, a point which is often lost sight of in horticultural experiments. In all probability the physical condition of the buds which are resistant to frost injury is approximately that of all buds during their normal rest-period. Up to the present but little light has been thrown on the nature of this or upon the actual modifications which occur upon the buds awakening from rest. Enzyme activity, conversion of carbohydrates and changes of protoplasmic permeability have all been considered almost as agents of the awakening process, but it is quite possible that a simple process conditioned by a rise of mean temperature will ultimately prove to be the cause of the renewal of activity at the end of the resting period.

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CHAPTER IX

FURTHER EXPERIMENTS ON THE RINGING OF WOODY SHOOTS

The works of Barker and Lees upon the effects of ringing and notching the shoots of fruit trees have been dealt with in a special chapter on account of their *ad hoc* nature. In addition, there is a formidable body of literature bearing only upon the relation of the practice to crop improvement or increase, but this has little interest in the present instance except in so far as it led later workers to investigate the modifications of physiological processes which followed such an operation.

The practice of ringing or girdling is an old-established one, especially in viticulture. Fuller (1911) ascribed its introduction to

a French viticulturist about 1745 when it was claimed that the result was not only a hastening of the maturity of the fruit but also an improvement of its quality. Fuller disagreed with the latter claim and said that, so far from being improved, the quality was generally greatly injured, although there was no doubt that the fruit did assume an appearance of ripeness earlier than normal.

The attention of horticulturists was for a long time directed solely towards the effect of ringing upon the production of fruit and the question of its appropriate seasonal employment. The point of view changed in time and in recent years investigations involving this operation have been chiefly directed towards an enhanced or controlled production of fruit-buds. Typical of such investigations are those of Drinkard (1915 and 1917) who found that ringing gave but small stimulus to fruit-bud development and this only when performed at a time when the leaves were fully developed. Together with other special practices he considered it unsuitable for employment in normal orchard procedure on account of the small knowledge possessed, which prevented the formulation of rules for their employment.

Results which were either inconsistent or difficult of interpretation had already been obtained by other workers with the vine, tomato and chrysanthemum, and Sorauer (1909, p. 778) summed up the position at the time of writing by declaring that ringing could only be employed advantageously in exceptional cases as an emergency measure the usefulness of which was often outstripped by its harmfulness.

Unexplained phenomena encountered by early workers with ringed shoots or branches were abnormal anthocyan formation in the leaves above the ring together with an earlier assumption of autumnal coloration and abnormally early leaf fall.

These and other consequences of modifications of physiological processes consequent upon ringing have generally been assumed to be explained by the well-known early researches of Hales (1748), Hanstein (1860), Hartig (1858) and Strasburger (1891), and to be summarised in the long prevalent view that the upward conduction of water is a function of the wood alone to which is confined exclusively the upward movement of carbohydrates in the spring, while a downward movement only is possible in the cortex and phloem. This latter flow was regarded as being checked by a ring with the result that the portion of the shoot above it received a larger supply of elaborated food during the growing season.

Bailey (1918), in discoursing on the ringing of vines, considered that a congestion of the parts above the ring was probable and indicated that these received extra nutrient at the expense of the parts below.

This prevailing view, that the ring was merely an obstacle to the passage of elaborated food material through the phloem, caused the importance of such elementary considerations as the actual physical loss of water by evaporation from the exposed surface of the ring to be completely overlooked. Sorauer (1909) alone dealt with the latter factor and ascribed the early assumption of autumn tints by the leaves above the ring to the relative poverty in water of the shoot portion there. Similarly, incomplete rings, where a strip of cortical tissue was left to bridge the gap, were generally regarded as incomplete barriers across which the flow of a small quantity of water was still possible.

An attempt to "determine more definitely whether the upward translocation of food takes place primarily through the phloem or through the xylem" was made by Curtis (1920) who made use of the ringing method for this purpose. In some cases ringing was accompanied by defoliation, but dormant shoots also were employed. He showed that both ringing and defoliation checked the growth of a shoot and an examination of his numerical data shows that, so far as growth in length is concerned, ringing had a more severe effect than defoliation. Curtis did not, however, separate the two effects, although their individual significance is apparent from the figures in question, but accounted for the check in extension growth by asserting that ringing prevented the upward passage of some substance or substances necessary to growth. He held that when leaves were present above the ring they produced enough of this substance to allow for continued growth in which case the check could not be due to lack of water. Ringed shoots which still retained their leaves always made fair growth.

In order of making growth his shoots could have been arranged in descending order of magnitude in the following classes: (1) Unringed—undefoliated, (2) Ringed—undefoliated, (3) Unringed—defoliated and (4) Ringed—defoliated: but occasionally the positions of (2) and (3) were reversed.

Curtis found that, under certain conditions, ringing was followed by withering of the parts above the ring especially when this was made near to the young tip and no leaves were left above it. This result agreed with those of Hanstein (1860) who found that ringed

willow cuttings placed in dry air showed withering of the phloem above the ring when leaves were absent from this portion. Lack of growth was ascribed by Hanstein to the absence of "newly assimilated sap" which was translocated through the phloem alone, but Curtis was unable to establish whether the check was due to lack of the food necessary for energy or building material or to lack of water arising from a deficiency of osmotically active substances.

Dixon and Ball (1922), in formulating a case against the adequacy of the phloem and cortex as a path for the downward translocation of carbohydrates, and for the wood being the channel by which the transport of these materials is effected, stated that it was only reasonable to assume that these will travel with the water current whether this moves in an upward or downward direction. They considered that Curtis, when criticising this view as the result of his ringing investigations, did not take into account the blocking of the tracheae which results from morbid changes spreading inwards from the injured region through the wood parenchyma and medullary rays.

In the course of an investigation of the factors governing bud formation the present writer found it necessary during the past year to carry out a number of ringing experiments on shoots of plum, pear, apple and cherry, both in the dormant and active condition, in order to determine the actual loss of water from the surface of the ring under different external conditions.

The shoots employed were apple, pear and cherry as uniform as possible in age, size and number of lateral buds. All were of the previous season's growth. Each shoot, after being cut to length, was mounted in a potometer of special construction which allowed of continuous observations being made of the rate of water uptake by the shoot. A duplicate potometer was set up as a temperature control.

For certain experiments the shoot was surrounded by a glass cylinder through which a stream of dry air could be drawn, while a blast arrangement permitted a stream of warm, dry air to be directed at the ring in cases where ringing was performed.

Even in unringed dormant shoots taken in December to February a slight uptake of water could be observed. As the time of bud-swelling approached the rate increased, becoming thirty to forty times as great when the buds had fully broken.

When the shoot was ringed to a width of 1–2 cm. there was an immediate increase in the rate of loss. If the hydrostatic pressure at the base of the shoot was increased this was immediately reflected

in a further increase in the rate of loss of water but water still continued to be taken up by the shoot when the pressure at its base was negative.

Nordhausen (1917 and 1921) showed that the leaves of a transpiring shoot exert a suction power which may produce a negative pressure in the shoot as great as eight atmospheres and which may be kept up for several days. He demonstrated that the active co-operation of the living cells was necessary to create the conditions under which this pressure could be produced, which emphasises the importance of taking this suction power into account when considering the mechanism by which water is raised in the shoot to the level of the ring.

When a stream of dry air was blown across the ring, the pressure at the base of the shoot remaining constant, the rate of loss of water increased greatly at once but returned to its original value when the blast was stopped, provided that the saturation of the surrounding atmosphere was unchanged. The rate varied rapidly with the saturation deficit of the atmosphere and in every case where the external conditions were modified there was a decided "lag," or "induction period" as the case might be, before the new uniform rate was taken up. Thus when the ring of a dormant shoot was enclosed in a glass sleeve the rate at once began to decline towards a very small value before reaching which both the surface of the ring and the inside of the sleeve became covered with tiny drops of moisture. On removal of the sleeve this moisture disappeared from the ring and the rate began slowly to increase towards its original value which was reached after a few hours.

The following example will give some idea of the quantities involved. A dormant shoot of plum was ringed in December in one internode to a width of 1 cm. It then showed a rate of loss of 0·04 c.c. per hour in the laboratory where the temperature was 17° C. and the saturation of the atmosphere 55 per cent. A loss of this magnitude must exercise a considerable modifying influence upon the ascending water current in the xylem whatever be the factors which actuate this. Observation of a ringed shoot during an experiment confirmed this, for if the buds were on the point of bursting those below the ring continued to make progress the whole of the time while those above the ring eventually became desiccated. Eight days were generally sufficient to cause this while two days later the cortex above the ring began to assume a shrivelled appearance. Actually the cells of the cortex began to lose their turgidity four or five days earlier,

the rigidity of the bark being sufficient to prevent shrinkage until their state of collapse was more complete.

As Curtis (1920) had reported for defoliated shoots the nearer the ring was made to the apex the earlier did the shrinkage appear.

The above results for dormant shoots may be summarised as follows. Loss of water by evaporation from the outside of a ring is often intense enough to set up a transverse flow of water there, in the xylem of the ring, the rate of loss being dependent upon the steepness of the gradient from the water column in the xylem to the external evaporating surface. Any condition which tends to increase the steepness of this gradient is accompanied by an increase in the rate of loss. The transverse flow may be so rapid as to disturb the supply of water to the portion above the ring and prevent an adequate supply from reaching this. Under these conditions a little growth of the buds above the ring is sufficient to exhaust the water from the neighbouring cortex with the result that shrinkage sets in, to be followed by complete desiccation of the cortex. The portion of the shoot below the ring is unaffected by these changes and its buds continue to make progress.

The state of things in shoots which had already begun to show active bud growth was found to be somewhat different. The loss of water from an unringed shoot began to increase as soon as the green tips of the lateral buds projected and this rate of loss could be again increased by varying the positive pressure at the base of the shoot. On being ringed in one of the lower nodes the rate immediately rose owing to the evaporation from the surface of the ring.

When it was desired to cut off the evaporation from the ring this was enclosed as before in a glass sleeve. By this means the rate could be decreased to a very small value although, owing to the continued progress made by the lateral buds, and the correspondingly increased loss of water by transpiration, it was difficult to obtain accurate differences for the evaporation value at different periods. The rate of loss from the ring appeared to diminish progressively with time, but as the transpiration value also decreased during the latter portion of the limited period of an experiment only a rough separation of the two effects was possible.

Normally the evaporation from the ring does not appear to be capable of raising water unaided. The continuity of the central water column is broken when the combined effect of hydrostatic pressure and transpiration lift falls too low and the diffusion stream becomes discontinuous. A set of experiments was therefore carried out to

determine more closely the relation between the rate of loss from the ring and that due to transpiration.

In these experiments the shoot was ringed low down and the buds below coated with vaseline. The portion of the shoot above was enclosed in a wide glass cylinder through which a stream of dry air could be drawn. This cylinder could be darkened by means of a sleeve of black paper.

The usual procedure was to determine for the unringed shoot the rate of loss in light and darkness respectively after which the shoot was once more illuminated until the original rate in the light was attained. The air current was then started and the procedure repeated. A return was thereupon made to the original conditions with the air at rest and the first set of observations repeated. By taking the respective differences for the ringed and unringed shoot the following values for the rate of loss from the ring were obtained.

Darkness—Still air	...	...	0·006 c.c. per hour
,, Air moving	...	...	0·012 ,, ,,
Light—Still air ...	...	...	0·016 ,, ,,
,, Air moving	...	...	0·019 ,, ,,

These figures establish generally that the rate of loss from the ring increases with the rate of transpiration and almost parallel with it.

When a shoot was ringed in two internodes it was found that the upper ring was more effective in increasing the rate of loss than the lower; but individual shoots differed widely in this respect.

Microscopic observation failed to show any such blocking of the tracheae, owing to morbid changes spreading inwards from the wound surface, as adumbrated by Dixon and Ball (1922). The wood of the ring was found to be completely dried out after evaporation from the surface ceased and the only mental picture which it was possible to form was of a breakdown of the lifting mechanism owing to the excessive demand made upon the ascending water stream by the evaporating surface of the two rings. As soon as the vertical column became discontinuous evaporation from the rings ceased.

This is, in all probability, the explanation of the fact that when a dormant twig is ringed drying out of the ring and shrinkage of the cortex above quickly follow; whereas in a shoot, the buds of which have already broken, transpiration is adequate to maintain a continuous water column for several days in spite of the competition for water to compensate for that lost at the surface of the ring.

That this procedure is independent of anything which might be connected with polarity phenomena, *e.g.* differences of conductivity or bud potentiality, was shown by an experiment in which a cherry shoot had the upper six nodes amputated after which it was placed in the potometer in the inverted position. The basal cut end was sealed with grafting wax after which the whole, with the exception of the three (now) lowest internodes, was enclosed in the wide glass cylinder. The three buds outside the cylinder were covered with a layer of wax.

The rate of transpiration was then determined for the unringed inverted shoot in sunlight and darkness respectively. After this the shoot was ringed in the third internode from the new base and the foregoing observations repeated. The ring was then enclosed as before in a sleeve of damp filter paper and the rate of transpiration determined under the same external conditions as at the start.

Observations of the rate of loss were discontinued on the fourth day, but the shoot was allowed to remain in the potometer for a further period of six days to observe the progress of the buds. During the first two days the two apical buds, which were of course at the bottom, went ahead of the rest and burst the wax coating which had to be renewed in consequence by a coating of vaseline. On the third day the basal buds, which were at the top, began to overtake the former and on the fourth day were making relatively better progress. This was maintained until the ninth day when a change took place in the four buds next above the ring. These had made little progress for two days and were now obviously drying out. The vaselined buds below the ring continued to grow out while good progress was also made by the buds near the new apex. In short, so far as its water relations were concerned, the shoot appeared to behave quite normally in the inverted position.

The practice is common in the orchard of employing rings which are only the width of a knife edge cut. Barker and Lees (1919) had made use of rings of this kind and experiments were therefore carried out to determine the loss of water by shoots ringed in this manner.

A shoot was set up in the potometer and, after the rate of loss had been determined in the steady condition, it was ringed with a knife edge ring in the third internode from the base. The loss from the ring was small and could not be measured precisely on account of the difficulty of depressing the transpiration of the shoot below a certain minimal value upon which the additional loss due to the ring could be superimposed in order to obtain a significant difference. Even

when enclosed in the glass cylinder, with the atmosphere saturated, a shoot would still continue to transpire for hours at a low rate.

If the shoot was transpiring freely before being ringed it was always found that the rate of loss of water was depressed immediately upon ringing.

This depression of the rate of water uptake was not shown by the shoots which were ringed to greater widths for the loss by evaporation from the ring was great enough to mask it.

Thus an apple shoot, which had been knife-edge ringed and was showing a uniform rate of loss of 0·121 c.c., responded at once when the width of the ring was increased to 8 mm. and showed a rate of loss of 0·134 c.c.

The primary aim of these ringing experiments was to elucidate the connection between the physical loss of water from the ring and any resulting modifications of the mechanism for supplying the portion of the shoot above the ring with water. It has been shown that only a partial separation is possible of the two effects due to evaporation from the ring and transpiration respectively. They are mutually dependent in so far as the rate of transpiration is modified by interrupting the continuity of the cortical tissues, while the continuity of the water column is an essential condition for evaporation from the ring to be possible. Whether this continuity is maintained or not depends upon whether the combined loss, due to evaporation and the utilisation of water by the portion above the ring, is exceeded by the amount raised and the results obtained confirm the conclusions of Nordhausen (1917 and 1921) as to the part played by the transpiration process in this connection. It follows, therefore, that many ringing experiments which have been explained entirely from the point of view of nutrition changes ought to be subjected to fresh interpretation after taking into account the above modifications of the water relations. That is, in a ringed shoot it is to be expected that the transpiration lift of the portion above the ring will be decreased; the portion immediately above the ring will as a consequence suffer an increasing poverty of water owing to withdrawal of its supply in two directions while the portion below will receive an abundant supply throughout.

With these principles in view ringing experiments were carried out by the writer (1922) during the Spring of 1922 upon trees of plum, apple, pear, and cherry. Some of the shoots worked with were pruned and it is necessary to consider the different effects separately seeing that very different results were obtained with the two kinds of shoots.

(1) *Pruning effect.* In most cases four to six internodes were removed from the distal end, the general ultimate effect being the stimulation of the three or four buds below the cut to more growth than they would have otherwise produced and to a greater extent than was possible by any other method. In the apple and pear the growth made by these buds was no greater than that which would have been made by the four terminal buds had these been allowed to remain; but in the plum the long succulent shoots produced by the buds below the cut exceeded both in size and average leaf-area those produced by the terminal buds of control shoots. In all varieties, pruned and unpruned, it was impossible to force out the two or three extreme basal buds into growth.

(2) *Wound-reaction.* An attempt to control this was made by cutting longitudinal slits down to the wood either above, below or behind buds in different regions of the shoot. No definite positive effect was obtained in any case. In other shoots a ring was made completely round the base of the bud and as close up to as possible without causing injury. A slight but irregular stimulating effect was obtained with buds of the middle region of unpruned shoots of pear and plum and pruned shoots of apple, the effect resembling that due to simple notching above the bud. As, in effect, the buds were notched both above and below it appears as though notching gives rise to a simple wound reaction, the common failure of buds which have been notched below being due to the injury which is easily caused by this particular operation. In order to produce any effect at the growing-point the stimulus must be intense, but increasing the intensity by decreasing the distance of the wound from the bud is limited by the possibility of causing injury to it.

(3) *Ringing effect.* (*a*) Ring close to the upper side of the bud: In pruned shoots of cherry there was a decided check to the four buds above the ring, that immediately above being the smallest. Their leaves were smaller and completely deficient in the red pigment characteristic of the normal young leaves. They were also more liable to become shrivelled owing to wind action. Below the ring the shoots developed in descending order of magnitude, that immediately below being approximately of the same size as one arising from the terminal bud of a control pruned shoot. As the season advanced and the ring healed the upper shoots began to make better progress and finally the apical three attained the same size as those below the ring. The shoot immediately above the ring, however, remained the smallest of all throughout.

In pruned shoots of apple and pear the effect was almost nothing, especially when the ring was made high on the shoot, but in unpruned shoots of plum the bud below the ring was generally as large as one of the uppermost while the bud immediately above was invariably the smallest.

(*b*) Ring close to base of bud: In pruned shoots of cherry the result was the same as in the previous case, but in pruned shoots of apple and pear not only was the bud just above the ring the smallest but in many cases it died away altogether. In unpruned shoots of pear no effect was obtained, while in similar shoots of plum the only effect was upon the bud above the ring which remained the smallest of all.

(*c*) Ring midway between two buds: Again the result was the same as in the previous case with pruned shoots of cherry and pear and with unpruned shoots of plum. Even in the unpruned pear shoots the bud above the ring was much smaller than those just below but generally speaking the response was not so great as in the plum shoots.

An explanation of all the above phenomena must take account of the initial check to the buds above the ring in pruned shoots in addition to the later progress of all the buds. For a considerable time after the buds above the ring have broken, the shoot-portion which bears them must be relatively poor in water owing to the loss from the cut end and the surface of the ring. At the same time the correspondingly poor development of the buds results in limiting the activity of transpiration as an aid in raising water. As the season advances this disability is gradually removed while the healing process puts an end both to the loss from the cut surfaces and the interruption of the cortical tissues at the ring. The buds above the ring are then able to resume their progress.

The small size of the bud immediately above the ring is no doubt due to the large loss of water in that locality during the early stages; the closer the ring is to the under side the smaller is the diffusion obstacle to this loss so that often the bud is completely dried out. In unpruned shoots the effect is relatively less, owing to the early development of the terminal group of buds and the correspondingly greater transpiration lift. In this case, with the exception of the bud immediately above the ring, the development of the buds follows an almost normal acropetal order of size.

It is generally accepted that response to ringing is only given to any extent by pruned shoots. Much of this response is probably due to the fact that the bud below the ring occupies the most favoured

position along the water-supply system and goes ahead accordingly. In other words, it is the conditions prevailing during the earliest stages of development that are decisive and a bud which is handicapped at the start does not ordinarily make up arrears when more favourable conditions are restored later.

The problem is complicated by the individuality or potentiality of the buds. There is a general acropetal order of potentiality which is independent of any process of inhibition activated during the early stages of shoot growth. It is probably inherent in the ontogeny of the bud which produced the shoot, for the three minute buds at the base of an extension shoot still fail to grow out when the conditions for the production of an inhibitor are removed by pruning down to them.

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CHAPTER X

SUMMARY AND CONCLUSIONS

In summing up the researches dealt with in the preceding chapters, and in attempting to define the present state of our knowledge of the factors which govern the formation and development of buds, we are called upon to discuss the results of the following three methods of attack.

(1) Direct observation of the normal growth cycle of the shoot and its buds and the relation of these to the external factors and to the character and position of the shoot.

(2) Regeneration experiments involving the wounding of the shoot and the study of its response to this treatment. In this category may be ranged all the investigations in which ringing, notching, pruning, inoculation or defoliation was employed.

(3) Investigation of the internal conditions in the shoot including studies of the sap concentration, food reserves or response of the shoot to treatment with chemical compounds as nutrients or stimulants.

It is necessary at the outset to bear in mind that experiments which evoke quantitative response on the part of the shoot need not necessarily throw much light on the causes of qualitative response such as is exhibited when flower-buds are produced instead of leaf-shoots and *vice versa*. Further, in attempting to account for response of both kinds it is necessary to keep in view the definite and well-known principles and phenomena of correlation which have been established as the result of the researches of Goebel, Klebs, Macdougal and von Vöchting (see *e.g.* Jost's *Plant Physiology*, English edition, Lectures 26 and 27). Starting from the postulate of Klebs (1903) that every organ laid down in the plant must develop if all the conditions of its growth are fulfilled we must conclude, inasmuch as the direction and magnitude of the development of an organ are correlated with that of every other organ, any disturbance of this correlation by the removal or stimulation of one organ will be followed by response on the part of the whole plant in the direction of a restoration of the original symmetry. Response of this nature is shown when the bud below a pruning cut grows out to replace the missing terminal. Howard (1920) found in *Indigofera arrecta* that the mode of branching of the roots corresponded closely with that of the shoot so that the type of rooting could always be foretold from the mode of branching, the root-system being the mirror image of the shoot. When actively growing plants were cut back drastically death of the fine roots invariably followed and extensive root regeneration was necessary before new shoots could be formed.

We have no knowledge of the internal causative factors responsible for this regeneration of symmetry nor is it possible to gain such from investigations of the first type. Even when it is established that a relation exists between an external factor and the quantity of formative response, we are still no nearer to an understanding of the

method by which the external factor acts upon the internal conditions or of the manner in which the latter exercise their formative action on, often, a few apparently selected growing points.

It may be taken for granted, therefore, that, so far as fruit trees are concerned, little will be added in the near future to that which has already been gained from methods of observation.

Two exceptions must however be made. It is often assumed that the individual buds have the same periodicity and that this is approximately the same as that of the shoot which bears them. In all probability this is not the case as a comparison of two widely separated buds on the same shoot will confirm. Their growth-cycles differ in respect of initiation, duration and the relation of external conditions to corresponding stages of development.

Consequently the orderly arrangement of the lateral buds on the parent shoot of last season's growth is as likely to be pre-determined by some common factor of periodicity, inherent in the buds, as it is to be governed by a factor released subsequently during the growth process of a particular bud.

Secondly, it is often assumed that the phenomena of bud development and growth encountered in the orchard are equally characteristic of all other trees and shrubs, so that the results of horticultural experiments are directly applicable to the latter. This is far from being the case; while even in fruit trees the differences between varieties, *e.g.* plum and cherry, are very considerable.

In both these cases there is room for more work of an observational and classificatory nature.

Much of what has already been said applies with equal force to investigations of the second type which, generally, merely add a few elementary manipulations to methods of observation. Up to the present there has been no separation of the effect due to injury —modification of the water relations, permeability and aeration and disturbance of the internal relations during the healing process— from that due to the actual loss of a plant portion, or the suspension of its function as the case may be. Naturally the horticulturist, by empirical methods practised over a number of years, may accomplish a good deal without any corresponding gain to the science of developmental physiology, but the result of his efforts will continue to emphasise how limited and unorganised past attempts at an exploration of this field have been. For example, in May, 1921, a bush apple tree which was about nine years old, and which divided above the ground to form a Y with two approximately equal branches, was

ringed to the wood in one branch just above the fork. In the Spring of 1922 the ringed half of the tree was covered with blossom, which later set abundant fruit, while the unringed half had only two flower spurs which set no fruit at all. Also the leaves of the unringed portion were larger and of a deeper green than those of the ringed portion, while its extension shoots were much longer.

Examples such as this serve to emphasise the inadequacy of our knowledge of the physiology of development, for here a simple operation released energies and modified development, both quantitatively and qualitatively, in a manner of which we have no comprehension. But so long as this is the case so long will the experimentation of the horticulturist continue to be uncertain and inordinately time-consuming, while the unsatisfactory state of knowledge of the problems of the orchard will remain unremedied.

Investigations of the third type promise to throw more light upon the manner in which the internal conditions direct or modify the formative processes in the plant, but those discussed in the previous chapters can only be regarded as pioneer efforts. Studies of both sap-concentration and food reserves are unanimous in indicating the need for more intensive and localised investigations, and for the employment of finer methods, before the connection between the internal conditions and the growing-point can be approached. Thus there is generally nothing in the complex of external factors prevailing during a current growing season to prevent the further development of the buds actually laid down during that season; and presumably the internal conditions can be made equally appropriate, for almost any of the lateral buds will grow out if its subtending leaf is removed in the middle of the growing season. If this removal is practised too early the bud cannot reach the point from which further progress is possible, while if the operation takes place too late the bud has entered its so-called rest-period from which it can only be forced by the application of an additional stimulus.

The rest-period appears, therefore, not to be necessarily bound up either with mean annual temperature relations or the variation in quantity or quality of the food reserves. Often one of two parallel effects has been viewed as the cause of the other and the possibility of a common factor responsible for both left out of consideration.

The inoculation experiments of Smith (1916, 1921) appear to fall into a class by themselves for they produced in the leaf drastic departures from the normal ontogenetic development resembling those which occur in gall formations. Evidently the protoplasm in

this case had undergone a complete change of character in order to produce these results which is an indication of the probable deep-lying nature of the factors which really govern the formation of buds.

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SOME EXPERIMENTS ON THE EFFECTS OF DRY AND MOIST AIR ON THE RATE OF RESPIRATION AND BREAKDOWN OF RIPE PEARS

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An investigation into the causes of the breakdown of fruits appears to be of primary importance in the discovery of suitable methods of storage. The work reported in this paper was undertaken to obtain some information on the subject with reference to pears which are liable to rapid decay. In view of the close relationship between respiration, transpiration and the physiological changes which take place in fruits, it was thought that a study of the influence of dry and moist air on these processes and their cumulative effect on the condition of pears might afford a clue to the manner in which deterioration occurs in them. Though the conclusions arrived at cannot be considered final, they may indicate the lines on which further work on the problem may be carried on.

Description of the Experiments

A. Respiration

Two series of respiration experiments were carried out on different dates. One upon "Carteret" pears and the other upon "Conference" pears. Both kinds of pears were obtained in the market and nothing is known of their previous history. Those which looked sound and representative were selected for the experiments.

The Carteret pears were already ripe, and the Conference pears which appeared mature became quickly softened during the experiments. In each experiment there were two sets of pears, one supplied with dry air and the other with moist air. The pears were placed in large jars and a constant current of air, freed from CO_2 by a strong solution of sodium hydroxide, was drawn through the apparatus by filter pumps, the rate being controlled by mercury pressure-regulators. In the case of the set in dry air, CO_2-free air was also passed through drying towers of calcium chloride. In the moist set saturated air was used at first but as it led to deposition of water on the fruit, with consequent development of fungal growths, the degree of saturation was reduced in subsequent experiments.

Estimation of carbon dioxide was made by the Winkler method. The CO_2 produced in each case was absorbed in a solution of sodium hydroxide, aliquot parts of which, after adding barium chloride in excess, were titrated with standard acid using phenolphthalein as indicator. Before starting absorption CO_2-free air was passed over the pears for 48 hours.

Experiment 1. Carteret pears.

In this case the experiment instead of being a continuous one had to be interrupted at intervals to remove the broken-down pears, being re-started with those which were still sound. The lots in dry and moist air are distinguished in the tables as *A* and *B* respectively. Pears of the *A* and *B* lots were kept separately throughout the experiment. Pears showing a breakdown of tissue were removed after each experiment and used for extraction of the juice.

Experiment 2. Conference pears.

Equal numbers of pears were put in eight jars, four of which were supplied with a current of dry air and four with moist. The pears were rubbed over with a dry cloth to reduce the chances of their becoming mouldy. The CO_2 from each pair of jars was absorbed separately and the pears after 13 days in the jars were removed for examination.

The respiration experiments were not carried out at constant temperature; the fluctuations of temperature were however small and the marked decline in the rate of respiration in both sets of experiments is quite outside the limit of any experimental error due to this cause. Pears were weighed at the beginning and at the end of each experiment and the loss in weight was noted in each case.

Determinations of the output of CO_2 were made after an interval of 16 to 48 hours in the case of Carteret pears, and 24 to 48 hours in

Conference pears. The amount produced was calculated in c.c. per 100 gms. per hour on the initial weight of the pears. Eleven sets of determinations, often of four each, were made in the experiments with Carteret pears, and seven of four each in the case of Conference pears.

The experiments were continued until the pears showed signs of rotting. The results are given in Tables I and II.

B. Transpiration

Loss of weight in pears was determined in the respiration experiments as well as independently. The results obtained with the Carteret pears kept in dry air and moist air respectively are indicated for each series in Table I. A set also of these pears was kept exposed to still air in the room where the experiments were being conducted. They were intended to serve as a control and it was interesting to find that their percentage loss of weight corresponded with that from the dry set. Of course the influence of the large space available in the room is to be considered and is not comparable with the conditions in the jars.

It was suggested that there may be individual variation in the loss of weight in different pears of the same kind. To obtain information on this point Carteret pears of Series III were numbered and their loss in weight taken separately. A marked variability was found in this respect among the various pears. To verify the results from the Carteret pears, and also to ascertain if there was any relation between the rate of loss in weight of a pear and the length of its life, fresh experiments were started on Conference pears. Twenty pears in sound and rather hard condition were chosen; they were kept exposed in a cool room and weighed at intervals until breakdown began, when they were cut up for examination and their condition, both external and internal, was noted as shown in Table VI.

C. Density of the juice

The specific gravity of the juice of pears was determined after the respiration experiment to get an idea of its concentration. Since the inner part of a pear is often more mature than the outer part, it was thought advisable to determine the two parts separately. The pears were peeled and divided into an external and a more internal portion. The pulp of each was cut up and enclosed in screw-topped metal cylinders which were then kept in a freezing mixture of ice and salt and left in a cold chamber for 24 hours. After thawing the

pulp was squeezed in a hand-press to extract the juice[1]. For purposes of comparison densities were also determined in a number of fresh pears. The division of a pear into an external and an internal portion being more or less arbitrary, a definite standard for comparison was provided by taking a mean by combining the densities with the weights of the pulp of the two portions. The results with the probable error of the mean are given in Tables III and IV.

D. Depression of the freezing-point of the juice

The freezing-point of the juice was determined by the Beckmann apparatus. The values obtained are shown in Table V. The observations were made on the mixed samples from the outer and the inner parts. In the table a comparison is made between the weight of sugar in each sample

$$\left(\text{calculated from its density by the formula } W = \frac{d-1}{0{\cdot}00386}\right),$$

the depression of the freezing-point observed and that deduced from the amount of sugar on the assumption that it is hexose. It will be seen that the difference between Δ observed and Δ calculated is approximately constant in each series, being 0·2 for Carteret pears and 0·15 for Conference pears. These differences represent molecular concentrations of the order of 0·01 per litre and are accounted for by the acid and neutral salts present in the juice. Such a comparison is of course only approximate but it serves to show that hexoses may form the principal sugar constituents of the juice of both pears throughout the period of examination and that it is unlikely that changes in the rate of respiration are due to the inversion of cane sugar.

E. Lenticels

Incidentally some observations were made on the lenticels which are to be found in large numbers on the epidermis of the pears. Their number was greater in the calyx region of the fruit. An average of 84 per sq. mm. was estimated from counts made on different parts of a Conference pear.

DISCUSSION OF THE RESULTS

In any interpretation of the results it is to be borne in mind that the pears were more or less fully ripened, and were purposely obtained

[1] Haynes, D. and Judd, H. M. The effect of methods of extraction on the composition of expressed apple juice and a determination of the sampling error of such juices. *Biochem. Journal*, 13. 1919.

in this condition as the object of the experiments was to obtain information regarding breakdown.

Examination of Tables I and II shows that during the first six or seven days of the experiment, the rate of respiration both in Carteret pears and Conference pears fluctuates somewhat, but towards the end an almost consistent fall is noticeable, the output of carbon dioxide becoming insignificant in the last period. It is important to note that the decrease in respiration coincides with the gradual softening of the pears which ultimately became much broken down. Special attention was paid to this point in the experiment with Conference pears and clear evidence of the same relation was found in these also.

Kidd, West and Briggs[1] have observed a "decrease in the respiratory index with age" in the various organs of *Helianthus annus*. The two phenomena appear to be closely associated with one another so that an investigation of the process of respiration should throw light on breakdown.

It will also be seen from the results with Conference pears that the amount of carbon dioxide produced was greater under dry conditions especially in the early stage of the experiment. Besides, as explained later on, dry air had a marked effect in accelerating transpiration and hastening the rotting of the pears. Anaerobic respiration in an atmosphere of hydrogen or nitrogen appears to prolong the life of the fruit[2] so the slowing of aerobic respiration may also give favourable results. Indirectly the present experiments tend to confirm this. In these cases in which aerobic respiration and transpiration have been intensified by dry air deterioration has set in considerably earlier. That the rate of respiration[3] was not affected to the same extent in Carteret pears may be due to the fact that they were more mature at the beginning of the experiment, while Conference pears were fast softening. Greater output of carbon dioxide in Conference pears even under moist conditions is attributable to their advanced stage of development.

The Effects of Transpiration

Tables I and II supply the figures of the percentage loss of weight and the respiration of Carteret pears of Series I–IV and also of

[1] Kidd, F., West, C. and Briggs, G. E. A Quantitative analysis of the Growth of *Helianthus annuus*. Part I. The respiration of the Plant and of its parts throughout the Life Cycle. *Proc. Roy. Soc.* B. **92**. 1921.

[2] Pfeffer, W. *Physiology of Plants*, 1, p. 537.

[3] Grove, H. C. Studies in fruit respiration. *U.S. Dept. Ag. Bur. of Chem. Bul.* **142**. 1911.

Conference pears. As was to be expected there is a distinct increase in the amount of water loss in all sets of pears kept in dry air; as compared with those in moist air, the rate was increased three to five times. The difference in the loss of water by dry and moist air has left a significant mark in the condition of the pears.

Carteret pears of the moist set kept much better than those of the dry set. The former lot retained the healthy yellowish-green colour while the latter were discoloured and in many cases were withered, wrinkled and turned brown. The pears of both the sets were cut in half and the internal condition of the thalamus was also examined. The peripheral tissues of those of the dry set were far more discoloured than those of the moist set. The more internal portions, however, did not show such a great difference. These results received corroboration from a similar behaviour of the Conference pears (Table VII). The conclusions indicate that the reduction of transpiration may be an important factor in the preservation of pears. As noted above, dry conditions would also promote respiration the acceleration of which would cause a rapid breakdown.

The results of the experiment regarding loss of weight in individual Conference pears are given in Table VI. It shows that the small pears lost more weight than the larger ones. This was of course expected as small pears have relatively a greater surface area. The largest pear weighing 105·7 gm. lost 6·8 per cent. while a pear weighing 69·5 gm. lost 9·2 per cent. in the same period. Moreover, the rate of transpiration went on increasing as the pears decayed. At the close of the experiment it was found that the pears which lost less weight were in better condition than those which lost more. The small pears became much shrivelled and the larger ones though comparatively turgid were softer. The latter condition is probably due to such pears retaining more moisture. Generally, the broad portion towards the calyx was more broken down and it is significant that a greater number of lenticels was found there.

After the experiment was over the pears were arranged according to the rate of loss of weight in descending order and the first and the last seven of the series were cut up and examined. The results are shown in Tables IV (*III*) and V (*c*) under *A* and *B* respectively. It will be seen from Tables III and IV that the specific gravity of the juice expressed from the outer part of the pulp was higher than that obtained from the more internal part. This difference is observed in the fresh pears as well as in both the dry and the moist sets of those in the respiration experiments. Greater concentration of the juice

in the outer part might be the result of its proximity to the transpiring surface and so subject to a greater loss of water. It may be that as the inner part is situated in the region of the developing embryo, organic substances are withdrawn more largely from its cells for the formation of seeds, etc. and thereby the concentration of the sap is reduced. Evidently the pears on removal from the trees have an initial difference in their osmotic equilibrium which persists afterwards.

The experiment showed further that the specific gravity of the pears which were most disorganized, was higher than that of those in better conditions. It seems, therefore, that a high concentration of the sap may be correlated with breakdown. Possibly some other factors also, such as intramolecular respiration, may be concerned in producing breakdown; and further research is needed to throw light on the matter.

A rough estimate was made of the weight of the residue left after expression of the juice, the residue was washed three times and after pressing out the liquid it was dried at 100° C. and weighed. It showed that in Carteret pears, though the specific gravity of the juice from the outer part was higher the weight percentage of the dried residue from the more internal pulp was greater. The difference was not so definite in Conference pears.

The results presented here are the outcome of a preliminary attempt which was made to study the subject rather on a general basis. A detailed investigation of the problem is being pursued by Dr D. Haynes from a biochemical point of view.

Summary

1. In experiments with pears ("Carteret" and "Conference") kept in moist and in dry air, the rate of transpiration is increased in dry air and also the rate of respiration in the case of Conference pears. The greater loss of weight in dry air is accompanied by wilting and earlier breakdown. In moist air, on the other hand, the pears maintained the healthy greenish-yellow colour and kept much better.

The rate of respiration falls off as the pears begin to show breakdown.

The specific gravity of the juice from the outer part of the fruit was found to be higher than that from the more internal portion in fresh pears as well as in those examined after exposure to dry and moist air in the respiration experiments. Specific gravity of the juice of pears as a whole was observed to be higher in the broken-down

condition than in those not yet over-ripe. Concentration of the sap is thus associated with breakdown.

2. Experiments on the density of the juice showed the presence of hexose sugars in the pears. A comparison of the observed depression of the freezing-point and that calculated from the amount of hexose sugars shows a constant difference, which may be explained by the presence of acid and salts in addition to the sugar.

In conclusion I wish to express my deep indebtedness to Prof. V. H. Blackman and Dr D. Haynes for the valuable help and advice which I have received from them.

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LONDON.

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TABLE I. Respiration of Carteret pears

Series I. (Oct. 11–17)

	No. of pears	Initial weight gms.	Final weight gms.	Loss %
A 1	19	1812	1762	2·7
A 2	8	689	652	5·2
B 1	19	1880	1865	0·7
B 2	8	681	674	1·0

c.c. of CO_2 produced per 100 gms. per hour (calculated on initial weight of Series I)

Date	Duration of experiment (hrs.)	Dry air		Moist air	
		A 1	*A* 2	*B* 1	*B* 2
12. x. 22	16	1·18	1·09	1·15	1·28
13. x. 22	18	—	1·08	1·17	1·14
14. x. 22	16⅔	1·40	1·32	1·40	1·77
16. x. 22	18	1·30	1·31	1·23	1·38
17. x. 22	22	1·32		1·30	

Series II. (Oct. 18–24)

	No. of pears	Initial weight gms.	Final weight gms.	Loss %
A 1	8	778	737	5·2
A 2	8	773	729	5·6
B 1	8	792	780	1·5
B 2	8	779	771	1·0

c.c. of CO_2 produced per 100 gms. per hour (calculated on initial weight of fruit)

Date	Duration of experiment (hrs.)	Dry air		Moist air	
		A 1	*A* 2	*B* 1	*B* 2
18. x. 22	42	—	1·24	—	1·31
20. x. 22	24	0·89	0·90	0·96	0·93
23. x. 22	48	0·89	0·85	0·87	0·95
24. x. 22	23	0·75	0·69	1·00	1·00

Series III and IV. (Oct. 25–30)

		No. of pears	Initial weight gms.	Final weight gms.	Loss %
Series III	*A*	8	761	747	1·8
	B	8	793	767	0·7
Series IV	*A*	8	747	730	2·2
	B	8	787	780	0·8

c.c. CO_2 produced per 100 gms. per hour (calculated on initial weights of fruit)

Series	Date	Duration of experiment (hrs.)	*A* Dry air	*B* Moist air
III	27. x. 22	24	0·85	0·83
IV	30. x. 22	48	0·53	0·76

TABLE II. Respiration of Conference pears

November 8–27

	No. of pears	Initial weight gms.	Final weight gms.	Loss %
A 1	5	384	327	14·8
A 2	5	422	352	16·1
B 1	5	400	383	4·2
B 2	5	409	392	4·1

c.c. of CO_2 produced per 100 gms. per hour (calculated in initial weight)

Date	Duration of experiment (hrs.)	Dry air		Moist air	
		A 1	*A* 2	*B* 1	*B* 2
8. xi. 22	48	2·74	2·44	2·16	2·14
11. xi. 22	42	2·58	2·39	1·33	1·36
13. xi. 22	48	2·17	2·01	1·49	1·21
15. xi. 22	24	1·68	1·91	1·43	1·25
22. xi. 22	24	1·33	1·17	1·25	1·38
23. xi. 22	24	0·81	0·68	0·83	0·85
27. xi. 22	78	0·72	0·56	0·56	0·65

TABLE III. Specific gravity of the juice of Carteret pears

Series I. Fresh samples (two pears in each sample)

Sample	Outside portion		Inside portion		Specific gravity
	Weight of pulp	Specific gravity	Weight of pulp	Specific gravity	Whole results calculated from preceding columns
1	71	1·0434	55	1·0419	1·0427
2	79	1·0395	51	1·0390	1·0393
3	88	1·0418	58	1·0414	1·0416
4	95	1·0420	55	1·0401	1·0413
5	86	1·0422	65	1·0413	1·0418
6	64	1·0418	59	1·0402	1·0410
7	75	1·0427	55	1·0409	1·0419
8	55	1·0423	55	1·0411	1·0417
9	70	1·0399	54	1·0393	1·0396
10	81	1·0430	66	1·0425	1·0428
				Mean	1·0413 ± 0·0003

TABLE III (*continued*)

Series II. Samples withdrawn from respiration experiment

(*a*) After Series I (nine pears in each sample)

From dry air	145	1·0411	145	1·0407	1·0409 ± 0·0009
From moist air	127	1·0406	183	1·0398	1·0402 ± 0·0009

(*b*) After Series II (eight pears in each sample)

From dry air	162	1·0447	175	1·0412	1·0428 ± 0·0011
From moist air	167	1·0405	161	1·0406	1·0405 ± 0·0011

TABLE IV. Specific gravity of the juice of Conference pears

Series I. Fresh samples (two pears in each sample)

	Outside portion		Inside portion		Specific gravity
Sample	Weight of pulp	Specific gravity	Weight of pulp	Specific gravity	Whole results calculated from preceding columns
1	114	1·0431	39	1·0415	1·0424
2	120	1·0436	37	1·0402	1·0427
3	97	1·0421	47	1·0412	1·0417
4	92	1·0410	44	1·0398	1·0406
5	122	1·0471	56	1·0423	1·0456
6	88	1·0421	36	1·0413	1·0418
7	103	1·0415	37	1·0394	1·0409
				Mean	1·0422 ± 0·0004

Series II. Samples withdrawn from respiration experiments of Nov. 16

1 5 pears from dry air. Loss of wt. 9·9 %	192	1·0429	97	1·0389	1·0415 ± 0·0002
2 5 pears from dry air. Loss of wt. 7·1 %	218	1·0437	75	1·0395	1·0426 ± 0·0002
3 10 pears from moist air. Loss of wt. 2·8 %	480	1·0393	206	1·0368	1·0385 ± 0·0002

Series III. Samples which had been kept in still air

A Loss of wt. 8·9 %	—	—	—	338	1·0433
B Loss of wt. 6·1 %	—	—	—	461	1·0402

TABLE V

Sample		Specific gravity of juice d.	Weight of sugar in 100 c.c. Calc. from sp. gr. o.	Depression of freezing-point Obs.	Depression of freezing-point Calc.	Difference
I. Carteret pears						
(*a*)	Fresh	1·0419	10·85	1·33°	1·12°	0·21°
(*b*)	*A*	1·0409	10·59	1·35°	1·09°	0·26°
	B	1·0403	10·44	1·25°	1·08°	0·17°
(*c*)	*A*	1·0423	10·96	1·35°	1·13°	0·22°
	B	1·0405	10·49	1·31°	1·08°	0·23°
II. Conference pears						
(*a*)	Fresh	1·0430	11·14	1·29°	1·15°	0·14°
(*b*)	*A* (1)	1·0419	10·85	1·22°	1·12°	0·10°
	(2)	1·0427	11·06	1·30°	1·14°	0·16°
	B	1·0391	10·13	1·20°	1·05°	0·15°
(*c*)	*A*	1·0433	11·21	1·32°	1·16°	0·16°
	B	1·0402	10·41	1·30°	1·08°	0·22°

TABLE VI. Loss of weight of Conference pears

Pear No.	Initial wt. Nov. 6 grm.	Loss % on previous weight: Nov. 10 grm.	Nov. 13 grm.	Nov. 17 grm.	Nov. 21 grm.	Total loss % of initial wt. in 15 days	Condition on Nov. 21
1	103·8	1·5	1·0	1·4	1·6	5·6	Firm, sound, colour yellowish-green
2	94·9	1·4	1·0	1·3	1·5	5·3	Firm, sound, as (1)
3	87·9	1·9	1·2	1·6	1·9	6·5	Soft at broad end, one decayed spot
4	91·02	1·7	1·1	1·0	2·2	6·0	Slightly softened
5	52·89	2·2	1·6	2·1	2·4	8·3	Much withered and wrinkled; softened
6	92·85	2·1	1·6	2·0	2·1	7·7	Withered and softened
7	80·3	2·1	1·5	1·5	2·3	7·6	Somewhat withered; one soft spot
8	91·15	1·5	1·1	1·3	1·7	5·6	Near calyx, soft; else firm
9	78·5	1·6	1·2	1·4	2·0	6·2	Firm, sound, colour yellow-green
10	60·9	2·6	1·6	2·2	2·4	8·6	Colour faded; broad part softened
11	105·3	1·8	1·4	1·5	1·9	6·5	Firm and sound
12	105·7	1·9	1·3	1·7	2·0	6·8	Broad portion softened
13	78·7	1·7	1·2	1·5	1·8	6·3	Slightly softened at broad portion
14	66·9	2·2	1·6	2·0	2·0	7·7	Surface wrinkled but firm
15	69·5	2·6	2·0	2·1	2·7	9·2	Colour faded; calyx region softened
16	76·1	1·8	1·3	1·6	1·8	6·4	Firm, sound
17	60·7	2·1	1·6	2·0	2·2	7·8	Colour faded; calyx region soft; surface wrinkled
18	98·5	1·5	1·1	1·4	1·5	5·6	Slightly faded near calyx, else hardly; yellowish-green
19	74·9	1·7	1·2	1·5	1·8	6·1	Soft near calyx; rest firm
20	85·32	2·4	1·6	1·9	2·1	8·0	Calyx region softened; colour faded

TABLE VII. Showing the effect of dry and moist air on the condition of Conference pears kept in jars and withdrawn at the end of the respiration experiment

Sample	Date	Dry air	Sample	Date	Moist air
G 5 pears	3. xi. 22 to 16. xi. 22	The pears turned brown. Calyx region softened in two pears. Stalk end shrivelled	*A* 5 pears	3. xi. 22 to 16. xi. 22	All pears hard and sound, colour greenish-yellow. Stalk end smooth and firm
H 5 pears	Do.	Colour changed to black. Three pears softened. Stalk end shrivelled	*B* 5 pears	Do.	Quite firm, colour greenish-yellow
E 5 pears	Do.	Two pears turned brown, surface withered and decayed. Colour of the remaining three pears, blackish. Stalk end shrivelled. Broader part more decayed	*C* 5 pears	3. xi. 22 to 27. xi. 22	Two pears firm and greenish-yellow. The rest somewhat softened but retain healthy colour
F 5 pears	Do.	One pear much withered and turned brown; two turned black and greatly decayed. The rest became yellowish-black	*D* 5 pears	Do.	Three pears firm and yellow. A large pear slightly softened but yellow. The rest somewhat softened at the calyx end, but yellowish in colour

THE CERAMIDIUM OF *POLYSIPHONIA*

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(With 14 figures in the text)

I PROPOSE to give here some account of the present state of our knowledge of the intimate structure of the cystocarp of *Polysiphonia* and allied genera, particularly as *Polysiphonia fastigiata* is likely to continue, on account of its accessibility, to be the species, by means of which the student is expected to make his first acquaintance with the Red Algae. Incidentally, I shall at the same time be affording myself an opportunity of acknowledging an error in one particular, into which I fell when I first interested myself in this subject many years ago.

To those who would like a broad survey of the advance of our knowledge of the group, I cannot do better than recommend the excellent "Historical Review of the Florideæ" by Dr Church in the *Journal of Botany* (1). I propose to confine myself here to the more

recent advance in our knowledge of the family Rhodomelaceæ, which includes *Polysiphonia* and its congeners.

A monograph on this family by Falkenberg appeared as recently as 1901. In this appeared figures illustrating the structure of the cystocarp in *P. sertularioides*, *Rhodomela subfusca*, and a species of *Dasya*, most of which have been reproduced by Oltmanns (8) in his *Morphologie und Biologie der Algen.*

I had already in 1895–6 published the results of some investigations of eight Rhodomelaceæ, viz. *P. nigrescens*, *P. fastigiata*, *P. violacea*, *P. thuyoides*, *R. subfusca*, *Chondria tenuissima*, *Dasya coccinea* and *Laurencia pinnatifida.* In 1906 appeared the remarkable paper of Yamanouchi (4) on *P. violacea.* I am not concerned here with the far-reaching nature of its cytological revelations, which made it a work of first-rate importance. It raised, however, some morphological issues which will be dealt with below. In 1911 Conolly (5) published an account of the structure of the cystocarp in *P. decipiens* and in 1914 Kylin (6) described, with cytological details, the cystocarp of *R. virgata.* Where, as the result of these researches, do we stand as to our knowledge of the minute structure of the ceramidium?

It may be convenient if I deal with the matter under three heads:

I. The derivatives of the pericentral cell.

II. The building of the wall.

III. The parasitism of the carpo-sporophyte (I adopt this convenient term from Church).

I. The Derivatives of the Pericentral Cell

The generally accepted view is, that the derivatives of the fertile pericentral cell in *Polysiphonia* are four in number:

(*a*) the 4-celled carpogonial branch;

(*b*) an inferior sterile branch, at first 1-celled, later 2-celled;

(*c*) a lateral sterile branch, at first 2-celled, later 4-celled; and

(*d*) the auxiliary cell in the superior position.

Of course it is not implied in speaking of (*b*) and (*c*) as sterile that they are in no way concerned with reproduction: as a matter of fact in many species they are wholly absorbed by the parasitic carpo-sporophyte. They are simply styled sterile in contradistinction to the carpogonial branch, which ends in the female organ.

With regard to the fate of the carpogonial branch, it is generally agreed that after fusion of the carpogonium with the auxiliary, what remains of the carpogonium and the three other cells are side-tracked and die off.

With regard to the growth of (*b*) from one to two cells, and of (*c*) from two to four, it seemed to me to occur as the result of the stimulus of fertilization. With this Conolly agrees as to *P. decipiens*, and Kylin as to *R. virgata*. Both the investigators also maintain for the species they examined, that the auxiliary cell is cut off from the pericentral above, after fertilization has taken place. Both Schmitz (7) and Falkenberg (2) had found the same thing in other species. I also found it so in several species including *P. violacea*. But Yamanouchi in *P. violacea* finds the auxiliary to arise in quite a different way. In the first place, he calls all the cells of both sterile branches accessories, which does not much matter, though it differs from the usage. He finds, however, that the lateral branch, which is described as 4-celled above, produces a fifth cell which inserts itself between the carpogonium and the pericentral, receives the diploid nucleus from the carpogonium and passes it on to the pericentral. This cell is clearly the auxiliary, in a special sense in which the term has been hitherto used, and the question is, Is it derived as Yamanouchi holds, from the lateral branch, or as all others hold, from the pericentral cell itself? The difference in the two views is brought out in Figs. 1 and 2; Fig. 1 is an adaptation of Yamanouchi's figure of *P. violacea*, and Fig. 2 is an adaptation of Kylin's figure of *R. virgata*. The dotted cell represents the auxiliary, the hatched cell the carpogonium.

I have moreover re-examined *P. violacea* itself, but am unable to find anything to change in my earlier description. Fig. 3 is a camera lucida drawing of the derivatives of the pericentral cell just after fertilization. Fig. 4 is the same without the carpogonial branch, Fig. 5 without the lateral branch. The material was treated with glycerine and stained in Hoffmann's blue. The cells stand further apart than they do in nature, but the pit-connections indicating origins are not adversely affected by the treatment. On the other hand it is difficult to trace pit-connections in microtome sections owing to the shrinkage in the wall after the severe dehydration. It is therefore safer to check the pit-connections made out in microtome sections, by resort to the glycerine treatment.

The thick-walled cell in Figs. 3, 4, 5 is the first cell of a gonimoblast filament.

In the re-examination of *P. violacea* I have found some variations which I had not recorded before. In the first place I found that the inferior sterile branch ran in one instance to three cells. In the same cystocarp the lateral branch ran to five cells, but no cell of the branch could possibly have been mistaken for the auxiliary, which occupied

the usual position, with a clear pit-connection with the pericentral, and which had budded off its first gonimoblast cell.

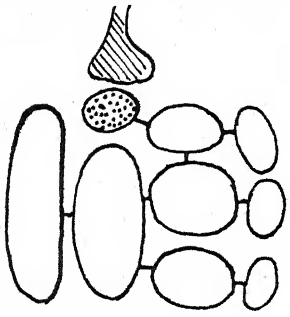


Fig. 1

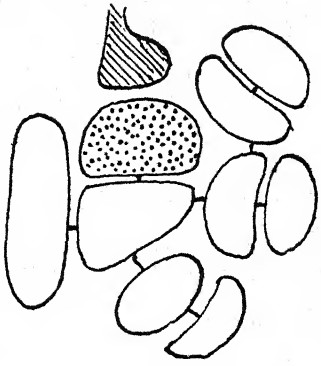


Fig. 2

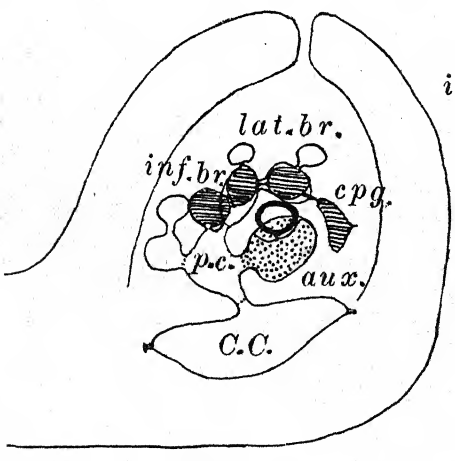
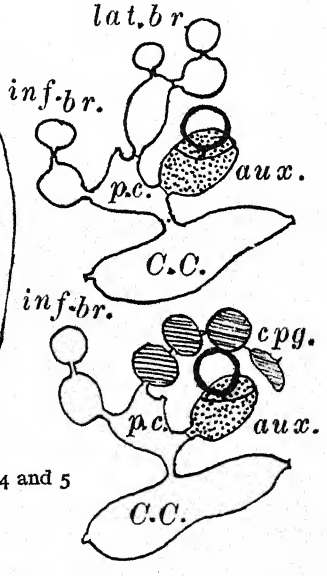


Fig. 3



Figs. 4 and 5

II. The Building of the Wall

To explain the way in which the wall of the ceramidium is built up, *P. violacea*, on account of its relative simplicity, will serve as a type.

At the first glance the wall of the mature cystocarp would seem to consist, over the upper three-quarters of its surface, of a single layer of cells arranged in fairly regular rows converging to the apical pore. Closer observation will discover, however, that these rows run

in pairs separated from each other by slightly thicker walls than those which separate the rows of the pair. In *P. violacea* I have counted 11 such paired rows. The two rows of each pair would seem to terminate at the stomium in a single cell, so that the stomium is bounded by 11 cells.

Focussing a little below the surface, there are seen spreading from the base, and converging to the apex, a number of chains of cells, whose contents differ from those of the wall proper by being more finely granular, so that they can readily be picked out by the eye. These chains would seem to run a straight course from base to apex (as straight, that is to say, as a curvature of the wall will admit) and to end just beneath the stomium. They will be found to correspond exactly with the 11 double rows of external cells referred to already and are so situate that they appear to run medianly beneath the partition walls separating the rows of each pair. They do not therefore in *P. violacea* form a continuous layer, but 11 chains separated from one another at the equatorial region of the cystocarp by considerable intervals. Further, the cells of the chains correspond exactly step by step with those of the paired rows. A broad pit connects the successive cells of the chains, and smaller pits connect each cell of the chain with the cells opposite to them of the double row. The odd cells of the stomium will be found on close examination to be the end cells of the internal chains, for they have a large pit-connection with the apparent end cell of the chain, but none at all, at first at any rate, with the wall-cells immediately below them. Fig. 6 shows the arrangement from the face, Fig. 7 in profile, and Fig. 8 combines the two views. Fig. 9 shows the appearance of the wall in transverse section. The cells of the chains are dotted as elsewhere.

When I first saw these internal chains of cells, I thought they sprang from the placenta independently of the wall, growing step by step with the wall and setting up secondary pit-connections with the cells of the wall. I spoke of them as paranematal filaments because it seemed to me that Agardh (10) had already seen, and even figured them, and called them paranemata. "In fructu juvenili *Polys. violaceæ* cellulas placentares plures vidi, a quibus exit fasciculus fertilis, constans exterioribus quibusdam filis sterilibus simpliciusculis—*paranematibus*—et interioribus filis brevioribus, pauci-articulatis et ramosis quorum in articulis ultimis gemmidia jam dignoscantur." He goes on to state that they seem to vanish later and they certainly are less easily observed in older ceramidia. But if these chains are

what Agardh really saw, and I cannot see what else it could have been that he saw, like myself he missed the clue which explained their true nature. This first appeared in Falkenberg's monograph, where at the same time my misinterpretation was pointed out. Oltmanns, Conolly and Kylin have all in turn directed attention to my mistake.

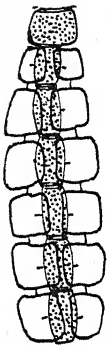


Fig. 6

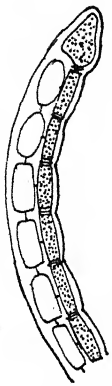


Fig. 7

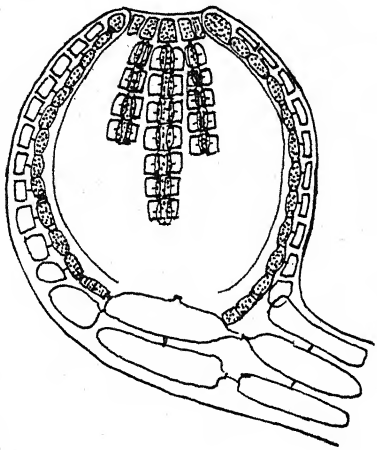


Fig. 8

Curiously, however, Yamanouchi has fallen into the same mistake as I did, although the nature of the threads had in the meantime been canvassed.

Now what is the explanation of the internal filaments in question?

Let us suppose that in *P. violacea,* the wall be regarded as formed of 11 concrescent shoots. A shoot in this species consists of an axial row and four rows of pericentral cells. Let us suppose that in the case of the concrescent shoots going to form the wall of the ceramidium, only two pericentral cells are cut off, and that only on the outside. The internal chains are the rows of axial cells of the concrescent shoots, terminating in the apical cell at the stomium. The paired rows are the "siphons" cut off, one pair from each axial cell. The correspondence in number and position between axial and pericentral cells, the single terminal cell at the stomium, and the pit-connections are all explained.

The internal chains therefore, so far from being an internal addition of the wall, are its very framework.

Figs. 10, 11 and 12 show the transition from the theoretical condition in 10 to the actual in 12.

Bornet (9), writing of *P. macrocarpa,* speaks of finding at Biarritz

a very curious monstrosity of this species: "Dans un échantillon qui était couvert de céramides, l'ostiole de beaucoup d'entre elles était prolongé en ramules plus on moins développés, garnis de poils, qui formaient au sommet de la céramide une couronne irrégulière." The figure which he supplies suggests that each concrescent shoot forming the wall, disengaged itself from its neighbours at the apex, and took on an independent growth.

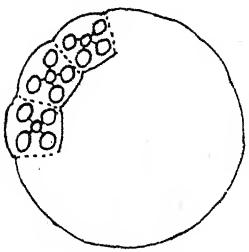


Fig. 10

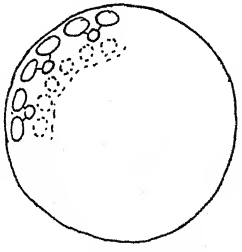


Fig. 11

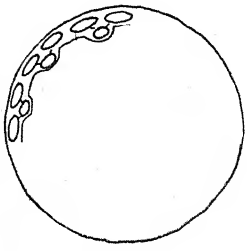


Fig. 12

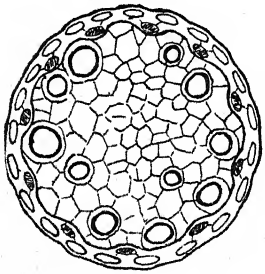


Fig. 9

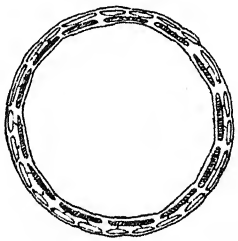


Fig. 13

Fig. 14

The appearance presented by these axial rows differs a good deal in different species of Rhodomelaceæ. In *R. virgata* itself it would seem that only one pericentral is occasionally cut off externally, leaving the mosaic of external cells less regular in the upper parts, as it is always in the lower.

Figs. 13 and 14 show a section of a cystocarp in *R. subfusca* and the face view of one of the chains.

In *C. tenuissima* I have described the chains as close-set laterally. When this is the case secondary pit-connections join the cells laterally, which is not the case either in *P. violacea* or *R. subfusca*.

When I described the chains as more than one cell deep in *L. pinnatifida*, the interpretation is that in the thicker wall, the pericentral cells take on also the appearance of filaments, the structure corresponding somewhat to that of the vegetative parts.

III. The Parasitism of the Carpo-sporophyte

The first step in the parasitism of the carpo-sporophyte is the fusion of the fertilized carpogonium (zygote) with the auxiliary. The next in the case of *P. violacea* is the fusion of the auxiliary with the pericentral cell from which it is claimed that it is derived. Even Yamanouchi (4), who bargains for another origin for the auxiliary, agrees that the pericentral cell is the next step in the fusion. Next all the six cells of the lateral and inferior branches are absorbed, then the central cell, and three or four of the basal cells of the 11 rows of central (axial) cells, so that in the end a mere shell of superficial cells is left, which are not included in the fusion mass—now consisting altogether of a couple of score of cells. After the first step, when the ooblasteme tube cuts across from the carpogonium to the auxiliary, the fusion is effected by the opening out of the pit-connections in retrogression to the central cell and then onwards along the other cells. When this mass is cut through, the incompletely absorbed walls are seen imbedded in the substance here and there, and the numerous nuclei are no doubt the progeny of the diploid nucleus of the zygote. Into the cavity of the cystocarp there bud out from the surface of this growing mass large numbers of branched filaments, the end cells of which become transformed into carpospores, which eventually emerge from their mucilaginous walls into the open. Fig. 9 shows a sketch of the contents of a cystocarp of *P. violacea* in transverse section, with the carpospores in part discharged, leaving empty spaces. The output of carpospores from each cystocarp must be very great, as they seem to be constantly renewed from the base over a considerable period.

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POLYTRICHUM PILIFERUM SCHREB., MUT. *PSILOCORYS*

By J. HEIMANS

(With Plate II and four figures in the text)

In May, 1910, the experienced Dutch mycologist Miss Cool and I were on a trip for collecting cryptogams, and we were much puzzled to find between Bussum and Hilversum (Holland) in small numbers a moss, which, though bearing ripe sporogonia, could not be identified with any generic type that might be expected to occur there. The epiphragma, peristomium and the ridges on the upper surface of the leaf, at once made clear that it must belong to Polytrichaceæ. The calyptra was exactly similar to that of *Atrichum* (*Catharinea*); the form of the sporogonium itself, on the contrary, did not at all resemble that genus, but had the outline of a *Polytrichum*-sporogonium, especially of that of *P. piliferum*, among which it grew in the sandy soil. Apart from *Atrichum* (*Catharinea*) there is only one European member of the Polytrichaceæ, *Psilopilum* Brid., of which one species only is known in arctic Europe (so far as I can find) that has a completely smooth hairless calyptra; but it was clear that our specimens could not belong to that species.

Some fifty fruiting examples were growing in one tuft among three times as many small fruiting shoots of *Polytrichum piliferum* (Plate II). The leaves and stem of the strange type were quite similar to those of the neighbouring *Polytrichum*; also the sporogonia could not be distinguished after the calyptra had been torn off.

The spot where the new plant grew was quite destroyed in the same summer in consequence of the construction of a new road. But in the summer of 1915 I happened to find again a small number of the same type about 2 km. from the first spot, but in quite a different locality, viz. under spruce-trees by the side of a path on an estate. Again they grew mixed with a greater number of *Polytrichum piliferum*, all in the same tuft. This time the leaves were fresher and consequently I could establish the absolute identity of stem and leaves, in outer appearance as well as in microscopic features, with those of the *Polytrichum piliferum* from the same spot; and no difference could be found between the sporogonia of the two types.

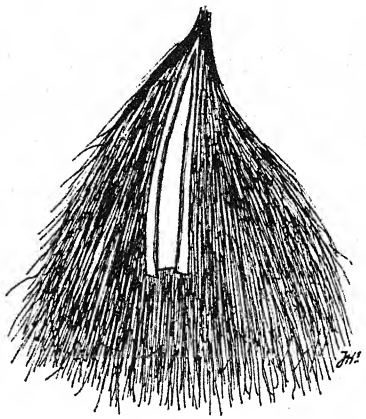


Fig. 1

Fig. 2

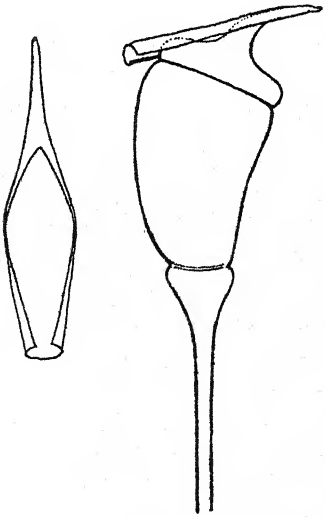


Fig. 3

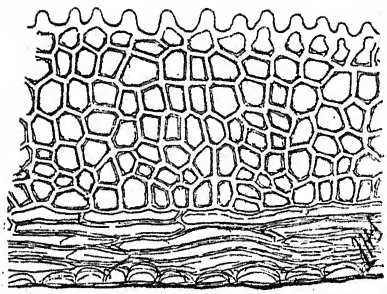


Fig. 4

Fig. 1. Calyptra of *Polytrichum piliferum* Schreb., showing the hairy covering laid out flat.

Fig. 2. One hair from the calyptra of *Polytrichum piliferum* Schreb.

Fig. 3. Sporogonium and calyptra of *Polytrichum psilocorys*.

Fig. 4. Side view of one lamella of the leaf of *Polytrichum psilocorys*, quite identical with the same part of *P. piliferum*, of which species this is the best distinguishing character.

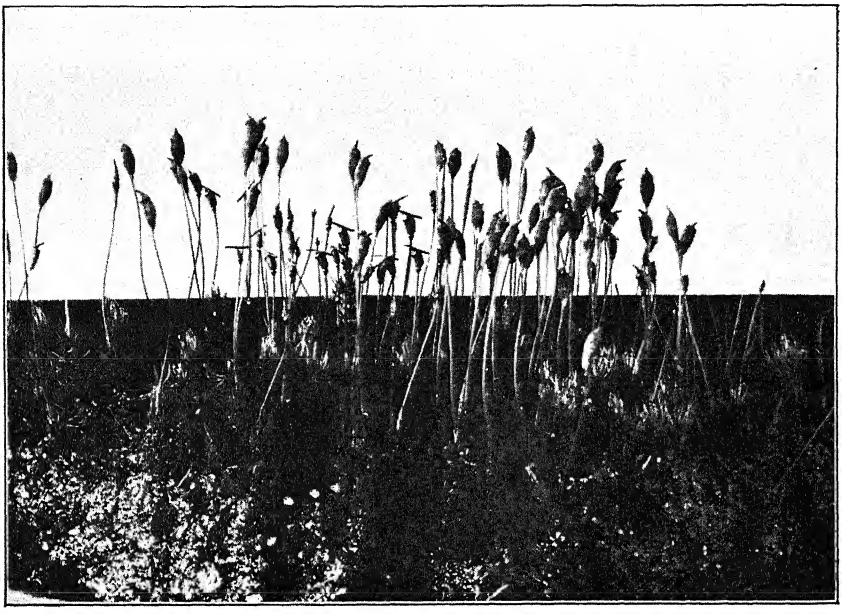
A careful comparison of the two so strikingly different forms of calyptra at once suggested an interpretation of the case. When the hairy cap of *Polytrichum* is lifted off, carefully torn open at one side and laid out (Fig. 1), it appears to consist of a cap of interwoven fringed crisp hairs (Fig. 2) which are inserted near the top of a smooth calyptra, which is slit on one side. This calyptra itself, without the hairy covering, is of quite the same form and structure as in *Catharinea* and as in the new form. In reality this form only differs from the typical *Polytrichum piliferum* in the one character of quite lacking the long obvious hairs on the calyptra (Fig. 3). Therefore it is most probably a sport, a mutation of that species, a loss-mutation (retrograde variety in the sense of Hugo de Vries) originating by the suppression of one unit-character of the species. Examples of such losses of one character are numerous enough, *e.g.* the peaches lacking the soft hairy down on the fruit, which are called "nectarines."

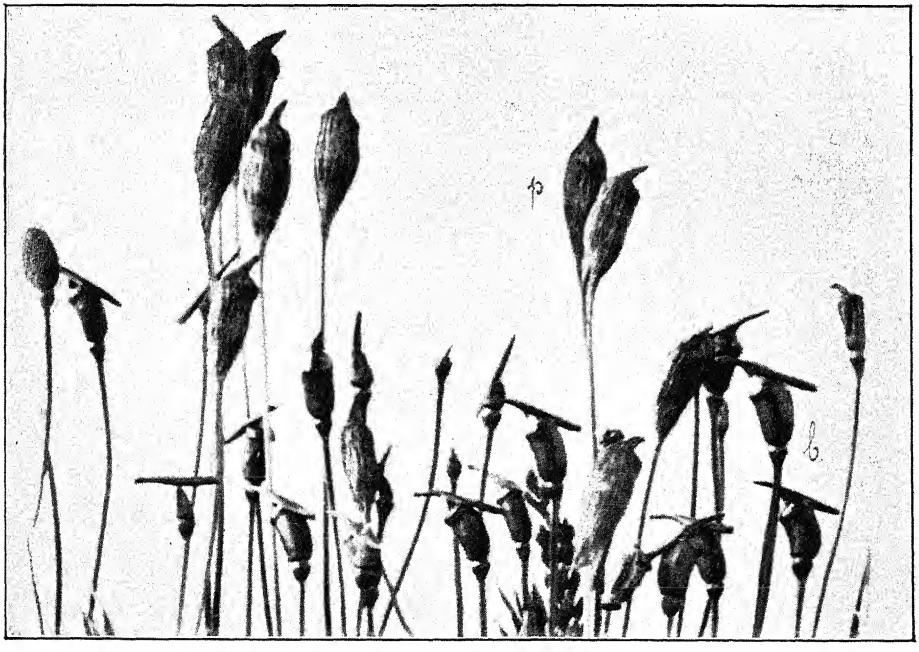
It is not especially peculiar, that an example of this kind of mutation should have been found among mosses, but the case is highly interesting because of the great change in outer appearance produced by this one loss, which happens to affect the most apparent feature of the plant, thereby suppressing the striking characteristic of the genus, from which the family has derived its name. In fact in *Polytrichum piliferum* the hairy coat of the calyptra is a more obvious character than in any other species of the well-known genus, covering as it does the whole sporogonium, fitting round the apophysis and even extending some way down around the seta. To such an extent is this heavily-coated calyptra the prominent feature of the species, that the outline of the sporogonium itself is not at all known to many people who are quite familiar with this very common moss of our sandy soils.

For this reason I believe that this new variety (for which I propose the name *Polytrichum psilocorys*) provides one of the most striking examples of such retrograde varieties that have hitherto been quoted in literature, much more striking even than the appearance of albinos in blue-flowering strains, or of smooth fruits in *Datura*, etc.

The photographs (Plate II) give an excellent idea of the two types as they grew together in one mixed tuft.

In order to investigate whether the new type was constant, spores were sown out on a strip of prepared peat ("entomological peat" on which insects are "set") soaked with Crone's food-solution,





Polytrichum piliferum Schreb. mut. *psilocorys*—growing mixed with the type in the same tuft. The lower figure shows a group of ripe sporogonia enlarged. p = type, b = mutant.

sterilized by boiling and kept in a glass box. They germinated abundantly and the culture remained pure. The young moss-plants appeared on the protonema, but they showed an extraordinarily slow growth and I never succeeded in getting any sporogonia from them, though they were kept alive for more than five years.

HORTUS BOTANICUS, AMSTERDAM,
August, 1923.

A NOTE ON THE PERIODICITY OF LEAF-FORM IN *TARAXACUM OFFICINALE*

BY B. MILLARD GRIFFITHS, D.Sc., F.L.S.

(With 14 figures in the text)

IN May, 1920, fruits were collected from a specimen of *Taraxacum officinale* found growing in the grounds of University College, Reading. The plant had large spathulate leaves which were about 50 cm. long and 11 cm. broad (Fig. I). It is not possible to state the age of the plant but subsequent evidence will show that it might be as much as three years old. Fruits of the plant were sown in a pot in the greenhouse of the Botanical Department, Armstrong College, Newcastle-on-Tyne, on April 13, 1921. By May 6 two seedlings appeared and these were planted out in the open in July. All the leaves produced were of the markedly dentate type (Fig. II), and continued to be so throughout the winter. In the spring, slightly larger, but still markedly dentate leaves began to appear (Fig. III). In May, 1922, the plants flowered (indicated by *F* in figures), and the leaves produced subsequently became less and less dentate, until by July, large, broad and spathulate leaves were developed (Figs. IV–VII). From August onwards the reverse process took place, until by the winter the leaves were once more of the markedly dentate kind.

The season of 1923 opened with the production of markedly dentate leaves as before, but they were of larger size (Fig. VIII). This type was maintained until the flowering period in April, immediately after which the form of the leaves began to change once more. From June to August, leaves of an increasingly less dentate form appeared, until finally they were broadly spathulate and over 40 cm. in length (Figs. X and XI). With the coming of autumn, the leaves began to decrease in size and increase in dentation, until by October they were

only some 12 cm. long and nearly as dentate as in the previous winter stage (Figs. XII and XIII).

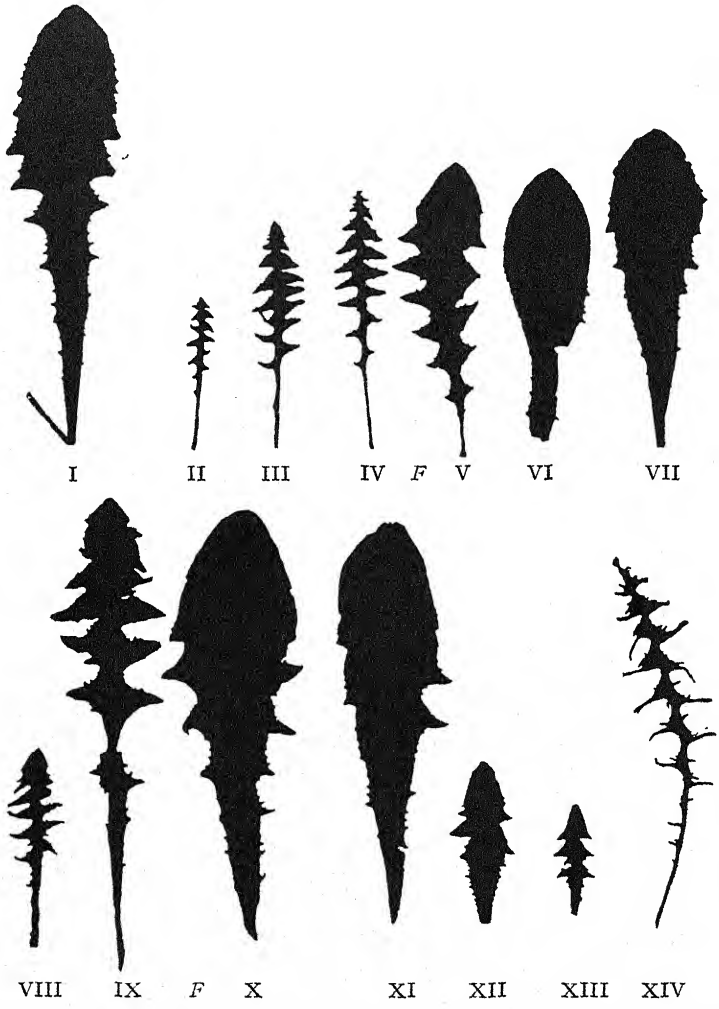


Fig. I. Leaf of parent plant. Figs. II–XIII. Successive leaves of filial plant over a period of two years, II–VII 1922, VIII–XIII 1923. Fig. XIV. Extremely dentate leaf from another plant. *F* indicates flowering period. The figures are from photographs of pressed specimens.

Unfortunately at this point building operations made it necessary to transplant the specimens, and serious damage was unavoidably done to the roots.

Growth experiments were also conducted on *T. erythrospermum.* Fruits were collected from typical specimens found growing in sandy ground near Reading, and also from the sand-dunes at Seaton Sluice, Northumberland. These were germinated and grown at Armstrong College, but it was found that as far as the experiments were carried, the offspring came true to type, and there was no change in leaf-form as in the case of *T. officinale.*

The periodic changes in leaf-form raise two questions, one of which is physiological and the other taxonomic. Priestly and Pearsall have shown that in the case of certain plants, it is possible to change the leaf-form by changing the pressure of the water-supply. When the pressure was increased, a young leaf which was normally lobulated, tended to develop into a less lobulated and more entire form, while diminution of water-pressure led to the lobulation of leaves which were normally entire (*Brit. Assoc. Report,* 90th Meeting, Hull, 1922, p. 394).

If the pressure of the water-supply is causative in the matter of leaf-form, *T. officinale* appears to be a species which shows large variations in its water-pressure throughout the year. On this interpretation, the pressure of water in the plant is low in the winter, and the leaves are consequently dentate, but after the flowering period, there is a progressive increase in pressure until the autumn, and the successive leaves are less and less dentate, until in the summer they become spathulate. On the same hypothesis, there is a rapid fall of pressure in autumn, and the leaves revert relatively rapidly to the low-pressure dentate form. It may be pointed out that the leaves of *Taraxacum* are very thin and watery, wilting takes place within a few minutes of cutting, and there is very little sclerenchymatous tissue. The rigidity of the leaf is maintained mainly by the turgor of the cells, and it is therefore not improbable that the leaf is peculiarly sensitive to changes in water-pressure.

The taxonomic aspect of these observations is also of interest because the form of the leaf is of importance in the division of the species into varieties. In Babington's *Manual of British Botany,* 10th ed. 1922, the varieties are: *T. officinale* Vill. Rchb., with leaves runcinate and broad; *T. laevigatum* D.C., runcinate pinnatifid with unequal teeth; *T. erythrospermum* D.C., runcinate pinnatifid with unequal teeth and intermediate smaller ones; and *T. palustre* D.C., oblong and entire sinuate-dentate or runcinate leaves.

One might suggest that possibly in *T. erythrospermum* we have a segregate of *T. officinale,* a segregate which has a water-pressure

variation-curve on one side of the original type mean, and that *T. palustre* is an analogous segregate with a variation curve on the other side of the mean. An extreme variation of *T. officinale* itself is seen in the case of the leaf shown in Fig. XIV, taken from another plant found in the grounds of University College, Reading. The dentation is extreme and resembles that of *T. erythrospermum* but on a much larger scale. Two attempts were made to germinate the fruits of this plant, but without success. No valid conclusion can be drawn from this failure, but it may be pointed out that if such an extreme variant is normally sterile, *T. erythrospermum*[1] may have arisen as a very rare fertile variant of the type, and that this variant had a low-pressure habit of water-supply, and was consequently able to colonize the sandy spots in which it now flourishes. As *Taraxacum* is an apomictic genus, the segregation of a variant is all the more likely.

DEPARTMENT OF BOTANY,
ARMSTRONG COLLEGE,
NEWCASTLE-ON-TYNE.

January, 1924.

THE CELL WALL IN THE RADICLE OF *VICIA FABA* AND THE SHAPE OF THE MERISTEMATIC CELLS

BY R. M. TUPPER-CAREY AND J. H. PRIESTLEY

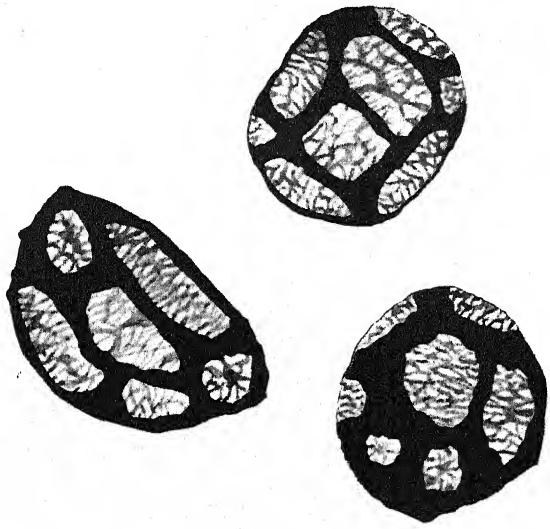
(With 1 figure in the text)

DURING investigations into the composition of the cell wall at the apical meristem of the root (1), it was noticed that when the cells of a dry radicle of the broad bean were macerated under suitable conditions, the walls showed a very definite and constant structure.

Sections of radicles previously soaked in water, then treated for 24 hours with concentrated ammonia, followed by 24 hours in

[1] *T. erythrospermum* also differs from *T. officinale* in the characteristic colour of the fruit. No suggestion is here made as to the nature of the correlation between this character and the leaf-form.

Eau de Javelle, macerated with comparative ease, for, as was shown in the earlier paper, the middle lamella in the broad bean radicle is probably a combination of pectin and protein, and maceration is therefore most readily produced by reagents which remove both these substances. If the cells are then tested with iodine and sulphuric acid for cellulose, the structure of the walls can be seen especially clearly in the large cells at the base of the radicle where it joins the cotyledons. Their appearance is striking; thick bands of cellulose run the length of the cell and are joined by similar transverse bands at intervals which give the whole the appearance of an irregular network,



while the intervening spaces are filled in by fine bars crossing from band to band and anastomozing with one another (Text-fig.). With iodine and sulphuric acid the structure is stained an intense blue, the thick bands very deeply, the intervening bars more faintly, probably because they are actually thinner.

The meaning of this arrangement remained obscure until attention was drawn to a demonstration by Lewis in a recent paper(2), that the typical shape of cells when "surrounded on all sides and compressed by similar cells," as in the Elder pith on which he worked, is a tetrakaidecahedron (*i.e.* the cell has 14 sides). Lewis reconstructed the cells by Born's wax-plate method from serial longitudinal sections, the models being made in wax after all the contacts had been studied and recorded. Figures comparing models of orthic tetra-

kaidecahedra with models of the actual cells, show a close approximation to the tetrakaidecahedral form and most deviations are shown to be due to a temporary change following cell division.

It has not been possible to determine the actual shape of the cells of the radicle, but the distribution of the cellulose reaction indicated in the text-figure suggests that the general form is tetrakaidecahedral, that the angles are occupied by thick bands of cellulose, and that these are connected by thin bars, possibly more slowly deposited, across the surface of the intervening protoplast.

This appearance of the cell walls after maceration is constant throughout the radicle in a varying degree. It is most marked in the cortical cells; the cells of the plerome have thinner walls and are longer in the direction of the main axis but the bands and bars of cellulose are still distinct; at the meristem itself the walls are extremely thin, the cells are very tightly packed together, and differentiation has probably not proceeded so far as in the rest of the radicle, but where maceration is complete a similar structure can be faintly seen.

Pectin stains such as Methylene Blue and Ruthenium Red show the same distribution as do cellulose reagents, and this bears out the assumption made in an earlier investigation (1), that in the cell wall of meristematic tissue, pectin is closely associated with cellulose. Macerated cells of dry broad bean cotyledons show the same features, while parenchyma cells from the hypocotyl of *Phaseolus* show a wall of cellulose evenly distributed, broken only by numerous small pits.

Whatever may be the nature of the intervening unstained spaces in the macerated cells, it must be remembered that in the unmacerated tissue this region, as also the space between the cellulose bands of adjoining cells, would be filled by a continuous matrix of protein and pectin from which the middle lamella of the differentiated tissue is ultimately developed. In these clear spaces, as cellulose deposition continues, there are left ultimately the pits in which protoplasmic connections are maintained through the otherwise continuous cellulose membrane.

This note on the description and interpretation of the cellulose deposits in the radicle of the broad bean appeared to the authors worthy of record, because, when the observation was first made, they were unable to interpret it, as the result of a subconscious assumption that the cells of the radicle were generally cubes and that the observed markings were on the surfaces of the cube. So far as

they are aware, the actual shape of the individual cells of the meristem of a growing point has been seldom, if ever, described. After re-examination of the question in the light of Lewis's paper, the authors feel no doubt that in the radicle of *Vicia faba* L. the cells are tetrakaidecahedral.

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(2) LEWIS, FREDERIC T. The Typical Shape of Polyhedral Cells in Vegetable Parenchyma and the Restoration of that Shape following Cell Division. *Proc. Amer. Acad. of Arts and Sciences*, **58**, No. 15. June, 1923.

ABNORMAL FLOWER OF THE HONEYSUCKLE (*LONICERA PERICLYMENUM* L.)

BY R. H. McCREA, B.Sc.

(With five figures in the text)

A PLANT of the Common Honeysuckle (*Lonicera Periclymenum* L.), bearing "double" flowers, was found in a hedge near Whitby in August, 1923.

The "double" flowers (Fig. 1) were much shorter than the normal ones (Fig 2), which were also borne by the plant (see p. 160).

The places of the stamens and the pistil were taken by some half-dozen lobed outgrowths (Fig. 3) which, together with the true petals, were coloured similarly to the petals of the normal flower. Moreover these metamorphosed organs were all traceable to the base of the flower and only on the posterior side were slightly epipetalous.

Some flowers were also present, on the same plant as far as could be judged from the thickness of the hedge, which had stamens (Fig. 4) of a type intermediate between the lobed petaloid stamens and true stamens (Fig. 5), but no pistil.

The Director of the Royal Botanic Gardens, Kew, has very kindly informed me that the above abnormality is an unusual one in this species, and that nothing quite agreeing with it is mentioned by Worsdell or other authorities.

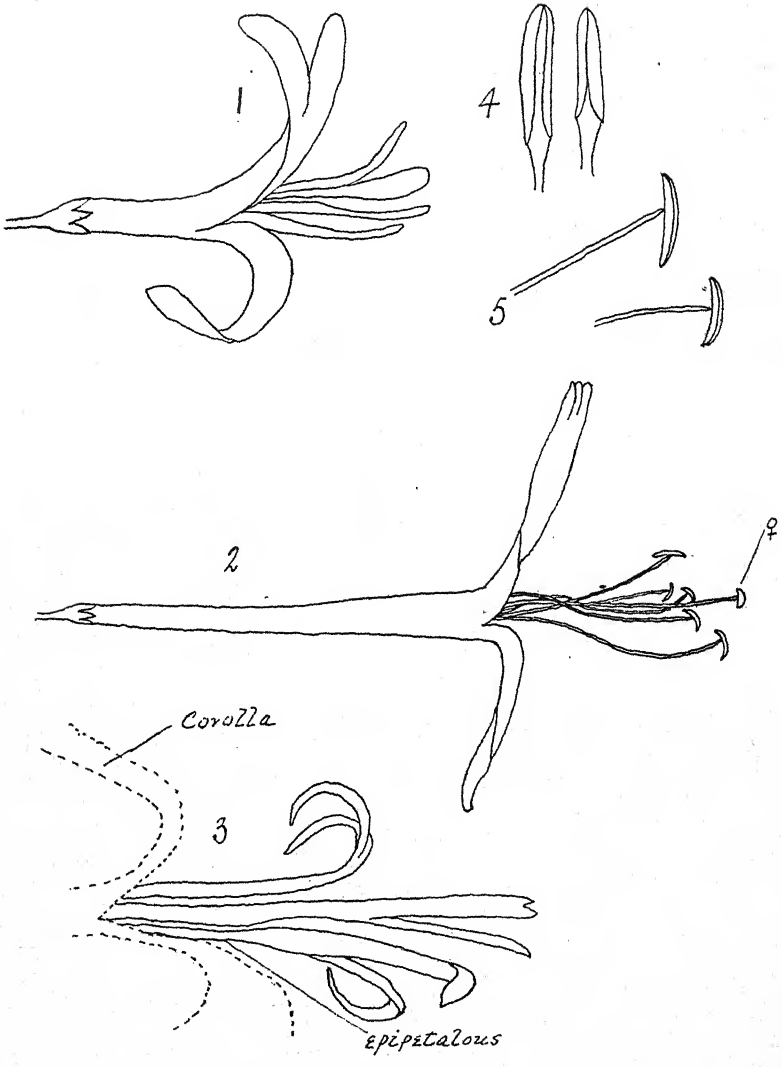

Fig. 1. "Double" flower. Fig. 2. Normal flower for comparison.
Fig. 3. Metamorphosed stamens. Fig. 4. Intermediate type of stamens.
Fig. 5. Normal stamens.

THE

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A STUDY OF THE ENDODERMIS IN THE FILICINEÆ

BY J. H. PRIESTLEY AND FRANCES M. RADCLIFFE

(With 12 figures in the text)

CONTENTS

INTRODUCTION

RECENT investigation having led to the conclusions that the structure of the endodermis has an important influence upon its physiological behaviour, and that this behaviour in its turn has an important reaction upon the further development of the plant, the writers commenced a study of this tissue within the Filicineæ, since in this group the endodermis is present throughout the plant, in root, stem and leaf, and appears at a comparatively early stage of development. The main aim of the investigation has been to accumulate data as to the structural and microchemical features of the various developmental stages of the endodermis and the influence exerted by

this tissue in the course of its development upon the exchange of water and solutes between the vascular system and the cortex. In the light of the data thus obtained a brief discussion is attempted of the significance of this layer of cells in the general development of the Filicinean sporophyte with its very characteristic adult structure and mode of growth. But as very little light has yet been obtained upon the factors leading to the development of the successive stages of the endodermis, it is not yet possible to make full use of these data in the service of causal anatomy.

Detailed work of this character cannot be extended over a very wide series of types, and the species mainly studied in the course of this work are *Dryopteris Filix-mas* Schott, *Pteridium aquilinum* Kuhn, *Nephrodium molle* Deav., *Pteris cretica* L., *Athyrium Filix-fœmina* Roth. and *Dicksonia* sp. A number of other species have been more superficially examined and the general conclusions arrived at hold for all. In every case the vascular cylinder is enclosed throughout its course by an endodermis which is as early in development as the vascular elements themselves. This endodermis usually passes through a series of developmental stages, with which the Marburg school of botanists, working under the guidance of Prof. Arthur Meyer, have already made us familiar; for the Filicineæ they have been described by Rumpf (22) for the root, and by Bäsecke (2) for rhizome and frond.

With this earlier work we are practically in complete agreement. The endodermal stages defined originally by Kroemer (10) have recently been described in work based mainly upon the Angiosperm endodermis (Priestley and North (18)) and the same general types of primary and secondary endodermis are met with in the Filicineæ. In agreement with Bäsecke and Rumpf, no tertiary stage endodermis has been found within the Filicineæ, but a more extended study of the secondary stage has shown differences in the structure from that shown by the same stage in the Angiosperms, and with this can undoubtedly be correlated the absence of the tertiary stage. It also becomes necessary to distinguish different stages of the development of the secondary endodermis because of their effect upon its physiological behaviour.

THE STRUCTURE OF THE ENDODERMIS

The two stages of the Filicinean endodermis, primary, with Casparian strip, and secondary, with suberin lamella, need separate discussion.

The primary endodermis. In agreement with Rumpf and Bäsecke, the tangential walls of the endodermal cells were found to give the cellulose reaction readily; the very narrow radial walls give the reaction of the Casparian strip alone. Text-fig. 1 shows the appearance of the Casparian strip, which stains red with phloroglucin and hydrochloric acid as if lignified. The appearance of a non-stainable margin of the strip is probably an optical effect due to the slight folding of the radial wall. The strip stains excellently with gentian violet after previous boiling of the section in 15 per cent. KOH, or warming in Eau de Javelle (Molisch (13)). With Nile blue sulphate a deep blue stain was obtained. The sections are soaked for an hour in Eau de Javelle followed by thorough washing in water until they are so free from alkali that no pink colour is observed on adding the Nile blue.

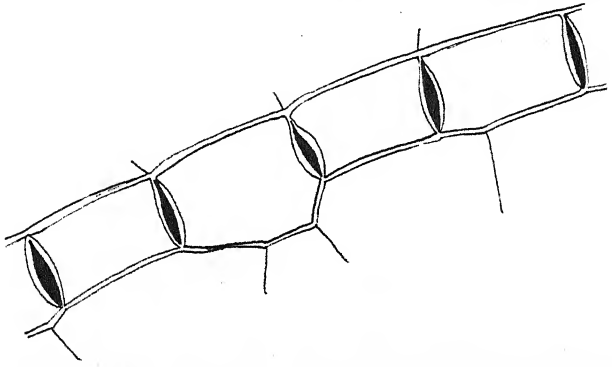


Fig. 1. Primary endodermis in transverse section in petiole of *Dryopteris Filix-mas*. Casparian strip present on radial wall. (× 1000.)

In the presence of the suberin lamella in the secondary stage, the Casparian strip stains differentially with di-methyl-amino-azo-benzene, the strip staining a vivid pink whilst the suberin lamella stains yellow. Sections to be examined in this reagent were soaked for 20 minutes or longer in an alcohol-glycerine solution of the reagent and then differentiated in aqueous 1 per cent. HCl (Mylius (14)). Methyl red, an indicator of very similar constitution (p. di-methyl-amino-azo-benzene-o-carbonic acid), also stains the Casparian strip red and the suberin lamella yellow.

The general conclusion drawn from these microchemical observations is that the Casparian strip represents the original cell wall, impregnated with lignin and with a fatty acid, retaining its acid properties, and therefore probably bound to the wall by its unsaturated linkage (Priestley and North (18), *loc. cit.* p. 122). The

evidence given later, p. 183, for the distribution of unsaturated acids in the developing stele should also be referred to.

Some maceration experiments carried out with pieces of endodermis scraped from the outer surface of the vascular bundle of the petiole of *Dicksonia*, throw further light upon the nature of the Casparian strip and are, therefore, briefly described here, although the experiments were performed upon a secondary endodermis. Left in Schultz's macerating fluid for from 4–6 hours, the Casparian strip dissolves away completely, the cells of the endodermis lying free from one another in the solution. Five hours' maceration in chromic acid (5 per cent.) also dissolves the Casparian strip completely; an alkaline oxidising reagent like Eau de Javelle has less effect upon the strip, but after 8–48 hours' maceration the strip becomes thickened and separates from the cells in places. It is noticeable that after such separation, if the material is treated with cellulose staining reagents, the whole cell wall gives the cellulose reaction and there is no indication of the former place of attachment of the strip. If iodine and concentrated sulphuric acid are used to develop the cellulose reaction, the Casparian strip gradually coalesces into drops of a yellow oil; after a long (48 hours') maceration in Eau de Javelle, this oil appears immediately the sulphuric acid is added. The fatty acid cannot be removed from the endodermis by boiling for half an hour in chloroform, and after such treatment the strip is as resistant as usual to sulphuric acid. Furthermore, the Casparian strip can be isolated from the secondary endodermis by boiling in 30 per cent. KOH for one hour, and then standing the endodermis in strong sulphuric acid. All the rest of the wall dissolves away under this treatment and the Casparian strip is left as the usual folded band (Text-fig. 2) which still stains yellowish-pink with di-methyl-amino-azo-benzene. If a piece of vascular tissue is left for 12 hours in Eau de Javelle, then for 12 hours in alcoholic HCl (25 per cent.), and finally for 12 hours in 0·5 per cent. aqueous ammonium oxalate, all the other tissues disintegrate into separate cells and vessels but the endodermis remains intact and can still be stained a pale pink with di-methyl-amino-azo-benzene.

We conclude on the basis of these staining and maceration experiments, that the Casparian strip consists of the ordinary cell wall, impregnated at a very early stage during differentiation with unsaturated fatty acid, which retains acid properties but has probably undergone some oxidation and condensation, as it is not removable by fatty solvents or by an hour's boiling in 30 per cent. KOH. This

fat-impregnated strip, while very resistant to alkaline hydrolysis, is less resistant to alkaline oxidising agents, and very readily oxidised by acid oxidising agents. Thus it contrasts with the suberin lamella, described below, and agrees with the behaviour of the Casparian strip of the Angiosperm (Priestley and North (18)). The impregnated basis would appear to be a middle lamella bounded on either side by a cellulose lamella; this middle lamella is not calcium pectate nor purely a pectin complex readily hydrolysed by acid to pectic acid.

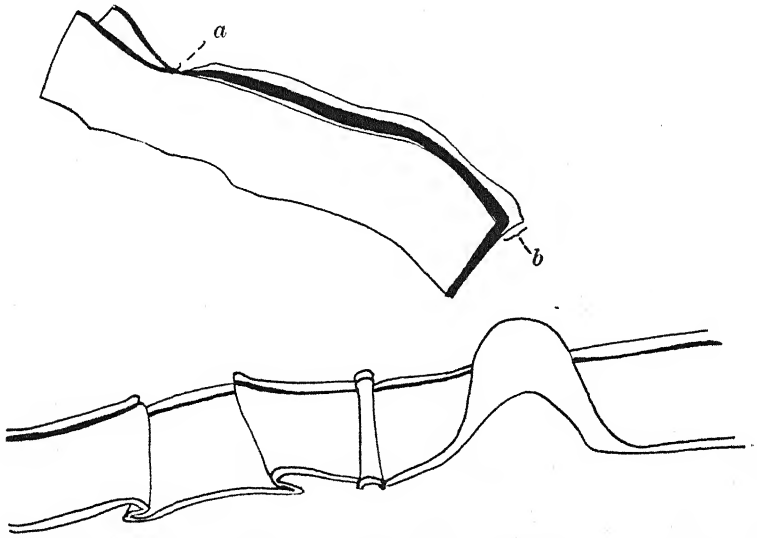


Fig. 2. Casparian strip from endodermis of *Dicksonia* rachis, isolated by previous treatment with 30 per cent. KOH, followed by concentrated sulphuric acid. The upper diagram shows piece in surface view, at *a* the strip divides, at a point which was formerly the end of an endodermal cell. Along margin *b* the appearance of the edge of strip is shown. Lower diagram shows characteristic folded appearance of strip. (× 1875.)

In view of previous work upon the Angiosperm wall during development (Priestley and Tupper-Carey (19)), it may provisionally be regarded as a pectin-protein complex; but there is little indication of the highly resistant nitrogen-containing basis to the strip found present in the Angiosperm, so that when the cellulose lamellæ are dissolved by suitable reagents, the residual substance fails to remain as an organised structure and the fatty substance coalesces into drops of oil. This appearance is only obtained in an alkaline oxidising medium which would decompose any protein present more rapidly than it appears able to attack the fatty substance of the strip.

The secondary endodermis. In the Filicineæ, as Rumpf and Bäsecke have already reported, two distinct types of secondary endodermis are to be met with. In one case the suberin lamella is continuous all round the wall of each cell; in the other the lamella only covers the inner tangential wall, extending over this wall and outwards as far as the Casparian strip upon the radial and transverse walls. *Dryopteris Filix-mas* shows an example of the complete suberin lamella, *Nephrodium molle* and *Pteridium aquilinum* have the one-sided lamella; in *Pteris cretica* and *Dicksonia* the lamella is also one-sided, but it is supplemented by traces of suberin as scattered granules or globules with the usual staining qualities lining the outer tangential wall. The same observation has been made by Rumpf who first reported the occurrence of this one-sided lamella.

In addition to these two types, the endodermis found in *Davallia* sp. will require some description, as in this case a definite suberin lamella was never obtained. Whether the lamella was complete or only over one wall, in every case examined it was found possible to distinguish three important stages in its development; but for the purpose of this distinction the necessary microchemical methods must be most carefully carried out. The suberin lamella gives the reactions of a neutral fat and does not give the lignin reaction with phloroglucin. Sudan III gives a very characteristic red colour with the lamella, but only after heating, and as the suberin lamella in its early stages readily melts into separate globules at the boiling-point of the alcohol-glycerine mixture used as medium for Sudan III, any observations made by the use of the reagent need very careful control by other staining reagents acting in the cold. Furthermore, the preliminary treatment with Eau de Javelle, often necessary with sections of ferns for reasons discussed later, has a very disintegrating effect upon the suberin lamella: in some forms (*e.g. Pteridium*) after a few minutes' treatment the lamella will no longer remain coherent on staining in Sudan III; in other types, such as *Dicksonia*, the lamella remains firm even after several hours' previous soaking. Stains used to obtain reactions without heating were di-methyl-amino-azo-benzene and methyl red.

In every case the first indication of the suberin lamella is the appearance of small granules or globules, lying along the inside of the cell wall or filling the lumen of the cells of the primary endodermis (Text-fig. 3). These globules are at first so small that they are only visible under a 1/12th oil immersion, but they gradually increase in size until they form an almost complete layer over the inside of the

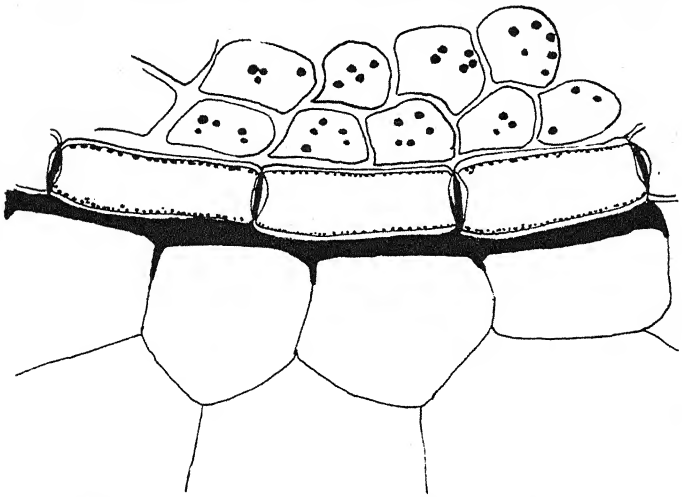


Fig. 3. Endodermis of petiole of *Dryopteris Filix-mas* in transverse section (× 1250), showing "granular secondary" stage, in addition to Casparian strip. Many fat globules are also present in the cells of the phloem and pericycle. The wall between the endodermis and innermost layer of cortex has begun to thicken.

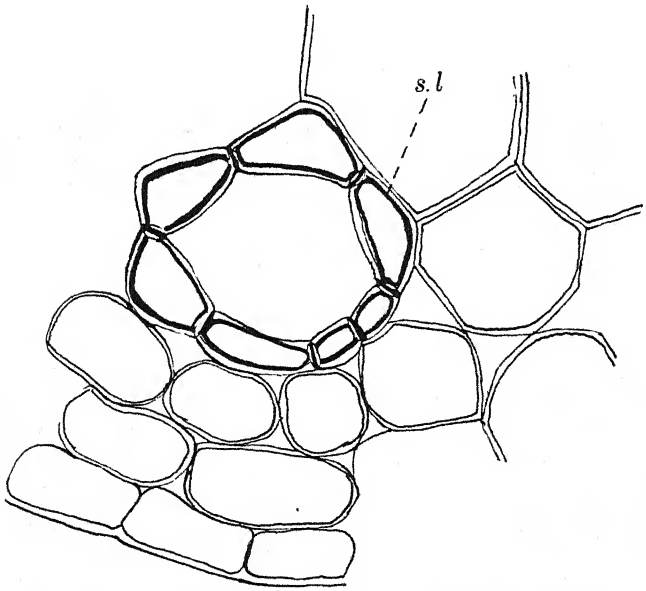


Fig. 4. Transverse section of the endodermis in leaf-vein of mature frond of *Dryopteris Filix-mas*, showing the secondary stage with a firm, continuous suberin lamella, *s.l.* around each cell. (× 1000.)

cell wall. This stage is found in the endodermis of young ferns or in the young growing region of older plants: it is a stage of considerable importance and will in future be distinguished as the "granular secondary" stage. Usually it passes fairly rapidly into a continuous semi-solid lamella, which obviously continues to harden with age; the endodermis in full-grown healthy plants is usually in this stage (Text-fig. 4). The last stage, found so far only in old and drying fern fronds, is characterised by collapse of the endodermis and the crack-

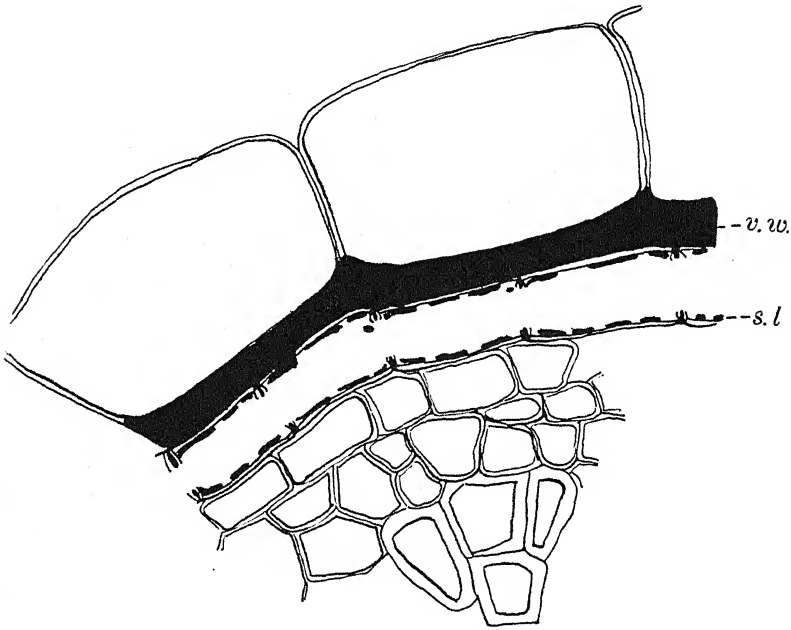


Fig. 5. Transverse section of the endodermal cells in a withered frond of *Dryopteris Filix-mas*, showing the broken suberin lamella, *s.l.*, and at *v.w.* the "vagin" impregnated thickened cell wall between cortex and endodermis. (× 1000.)

ing of the hard dry lamella (Text-fig. 5). Maceration experiments with strips of endodermis show that the suberin lamella disappears rapidly in chromic acid or in Eau de Javelle, in Schultz's macerating fluid it appears to be more resistant than the Casparian strip, so that the cells are isolated after short maceration but still retain a suberin lamella. In the earlier study in this laboratory of the secondary lamella of the Angiosperm, and in further studies of the Angiosperm lamella that have been in progress concurrently with the investigation whose results are here presented, these three stages in the lamella

have never been detected, and their absence is undoubtedly to be correlated with the presence in the Angiosperm of a basal substance in which the fatty substances are held and which is deposited contemporaneously with the fat. The evidence is convincing that no such basal substance is ever present in the secondary endodermis of the fern. If sections containing a secondary endodermis are warmed for a few minutes in Schultz's macerating fluid, washed in water and then boiled in potash, the suberin lamella is completely removed. The remaining walls of the endodermis, with the exception of the Casparian strip, then stain blue in chloriodide of zinc, the suberin having completely disappeared or remaining as yellow globules within the cells.

The most careful control of this reaction, together with the observations of the successive stages in the formation of the lamella, leaves no doubt that the lamella consists purely of a layer of fatty substance deposited upon the inside of the original cellulose wall, but unaccompanied by any other substance capable of remaining as a lamella after the fat is removed.

We have here also the explanation of the absence of any tertiary endodermal stage. In the Angiosperm, simultaneously with the deposit of the fat of the future suberin lamella, there is proceeding the deposit of a cellulose-like substance (Priestley and North (18)), which remains as a lamella even if the fat is removed by saponification; when fat deposition ceases, cellulose may continue to form and a cellulose lamella then becomes visible within the suberin lamella. In the fern in no case is any cellulose deposited during the formation of the suberin lamella: hence the latter is without a resistant cellulose basis and the tertiary stage of the endodermis never appears.

As the fatty substances gradually accumulate upon the wall, they fuse into a continuous layer, then harden, dry and crack, with the result that in the fern, unlike the Angiosperm, the three stages of development described above are met with. These three stages will obviously have very different effects upon the exchange of water and solutes between vascular system and ground tissue, and it is important therefore to realise their distribution in the plant before physiological experiments are discussed.

Throughout these investigations, very few evidences were found of the presence of passage cells within a secondary endodermis, the only clear cases being lateral passage cells in the veins of the pinnæ of the fronds, *e.g.* in *Polystichum angulare* Prest. (Fig. 10) and *Dryopteris Filix-mas*. These passage cells are only temporary, the whole endodermis being blocked in mature fronds.

THE DISTRIBUTION OF THE ENDODERMIS

The primary endodermis is continuous around all the vascular tissue of every fern examined with one exception (*Pteris cretica*), and the secondary stage has always been preceded by a primary.

Rhizomes. Hofmeister(7) distinguished two types of fern, the male fern type with bulky upright rhizome and radial arrangement of fronds and the *Pteridium* type with comparatively slender creeping rhizomes, from which the fronds arise laterally at considerable intervals. Bäsecke terms Hofmeister's first type the "storage" rhizome and points out that this type of relatively fleshy rhizome is characterised by the permanent retention of a primary endodermis around the vascular system, a secondary endodermis appearing in the frond but usually only above the swollen leaf base.

This distinction appears to have some significance, but its full meaning is obscure and all fern rhizomes do not fall naturally into one of the two simple categories. It is interesting to note that a species of *Davallia* which seemed to be bulky and juicy enough to rank as a storage rhizome, although running horizontally and bearing leaves at relatively distant intervals, also possessed an endodermis which failed to fall definitely into either category: fat globules had accumulated to a considerable extent within the protoplasts of the primary endodermis, but instead of lining the walls as a lamella they formed a relatively dense but incoherent mass within the cell.

The development of the secondary endodermis has been carefully followed in the rhizome of *Pteridium aquilinum*. The growing apex is densely covered with hairs, but the first hand sections taken below these hairs show no differentiation: the whole area is meristematic, the cells giving a pink colouration with Sudan III throughout, indicating the presence of fats both in walls and contents. In about the eighth section thus cut by hand the first signs of lignification are visible in the vascular bundles, and simultaneously the Casparian strip appears. In the next two sections the endodermis shows a very clear and definite Casparian strip and also minute globules of fat are visible on the inner tangential walls. In succeeding sections this fatty deposit increases and within about 2 mm. of the apex the complete internal suberin lamella is present.

Fronds. In the frond, in all cases examined, a suberin lamella ultimately develops and passes through all its stages, but it is not necessarily completely developed so close to the apex. On the contrary, if young developing fronds are examined just as growth is

starting in the spring, the primary endodermis appears as soon as the vascular tissue begins to differentiate in the young fronds of *Pteridium* before they appear above ground, but below the region of this the secondary endodermis remains in the incomplete granular state right down to the place of origin of the frond upon the axis. The young frond of *Dryopteris Filix-mas* is in the same condition in the Spring until it begins to uncurl. Then, in all cases, as the frond grows, the complete secondary endodermis rapidly follows the deposit of the fatty emulsion on the endodermal wall. As the endodermis is thus completed the petiole often changes in colour at the surface, becoming browner and its tissues drier and firmer.

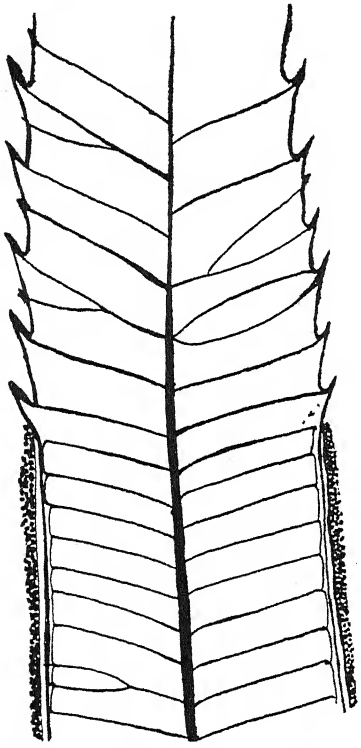


Fig. 6. Diagram showing venation and margin in part of pinna of *Pteris cretica*. Beneath the sorus there is a vein parallel to the entire margin, in sterile portion of pinna, marginal vein is absent and lateral veins terminate in the marginal teeth.

In the leaf blade the endodermis also rapidly passes over into the complete secondary stage, except at two places—the base of the sorus and the tip of the vein, as already pointed out by Bäsecke. The endodermis in these two regions is sometimes primary but usually in the incomplete secondary stage. It is in this stage below the base of the sorus in *Dryopteris Filix-mas*, *Pteridium* and *Nephrodium molle*, and not until the spores have ripened does the complete secondary endodermis form in this region. In any frond of these types, if a section is cut through the vein at a similar level but where no sorus is present, the complete suberin lamella will be found present at a very early developmental stage, although it may readily melt under too severe heating in the Sudan III reagent.

Pteris cretica showed an unusual condition. Fig. 6 shows the outline of a portion of the lamina: the midrib of the young leaf, like

the petiole, contains a complete secondary endodermis, fairly resistant to heat, while in the lateral veins the suberin lamella is still continuous though more readily melted. The sporangia line part of the margin of the pinna, and beneath them lies a vein. This vein does not run along the margin when the sporangia are absent, and in such regions the margin is serrate, although entire in the neighbourhood of the marginal sorus. If a transverse section is cut across the marginal vein (Fig. 7), a complete secondary endodermis is seen to be present upon the dorsal side but fails entirely in the region beneath

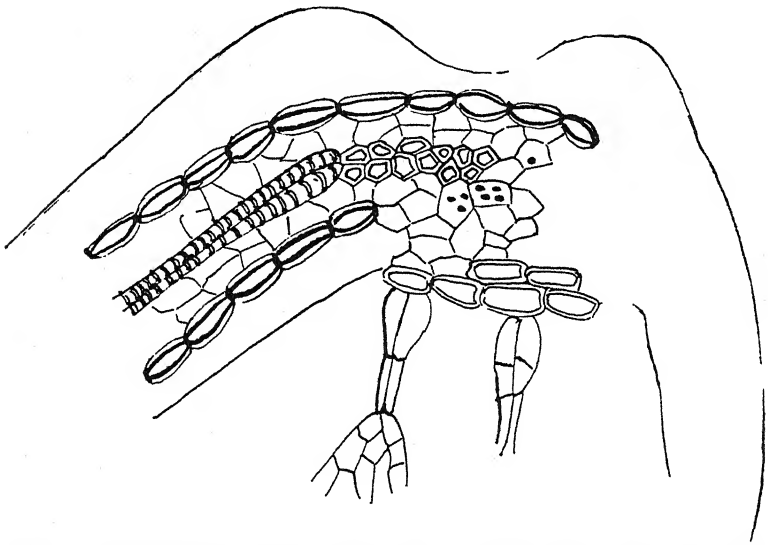
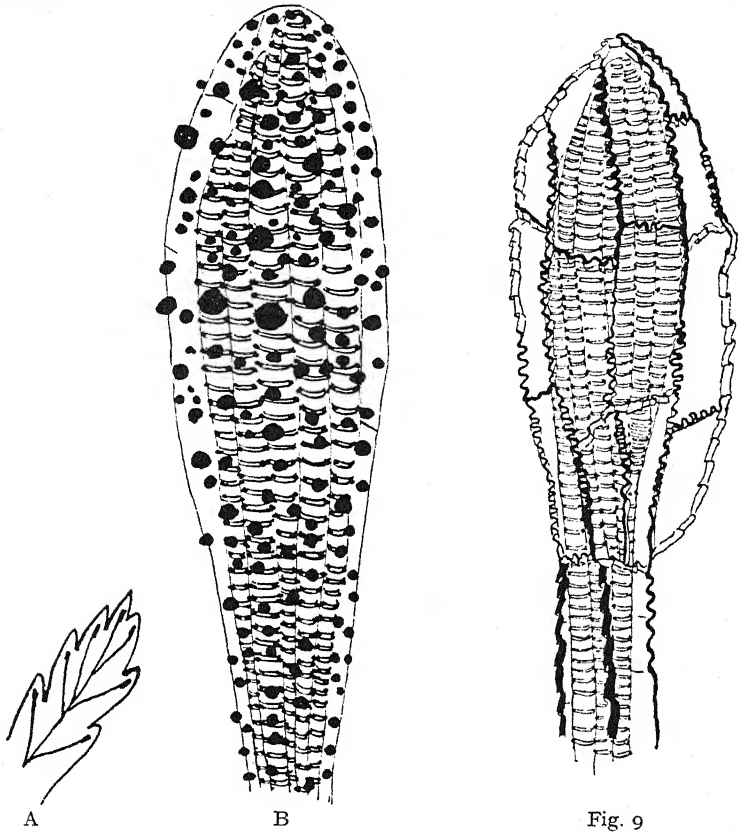


Fig. 7. Transverse section of marginal vein running below sorus in *Pteris cretica* (× 800). The endodermis is interrupted between the vascular elements and the base of the sorus, the black dots within some of the cells indicate the presence of globules giving fat stains.

the sorus. The primary and secondary stages of the endodermis develop almost simultaneously around the upper half of the bundle: on the other side the cells contain globules of fat, seen very clearly on staining with di-methyl-amino-azo-benzene, but show no Casparian strip and no suberin lamella. In the frond of *Pteris cretica* no indication has yet been found of the secondary endodermis ever completely enclosing the bundle, but it is worth remembering that this fern has only been examined when growing under greenhouse conditions and that in all ferns so far studied in their normal habitat complete closure of the endodermis ultimately occurs (Bäsecke(2), *loc. cit.* p. 38).

In *Dicksonia* the sori are marginal and the veins of the frond, which have a completed secondary endodermis, continue to the base of the sorus, opening into this. In types without marginal sori, as *Dryopteris Filix-mas* and *Nephrodium molle*, the vein tips are more



A B Fig. 9

Fig. 8. A. Portion of pinna of *Dryopteris Filix-mas*, showing how vein tip terminates in hydathode.

B. The hydathode, after macerating in Eau de Javelle and then boiling in Sudan III in alcohol and glycerine. The rounded fat globules are stained red. (× 300.)

Fig. 9. The hydathode of *Dryopteris Filix-mas* after maceration in concentrated sulphuric acid. Around the central core of xylem a network of Casparian strip still persists. (× 300.)

readily examined. The vein has a swollen club-shaped ending pointing into a serration and reaches nearly to the margin of the fronds (Fig. 8 A). Transverse sections of the club-shaped region show globules of fat in the endodermis and those which include the exact

top of the vein show simply a mass of cell with fat deposits. Examination with di-methyl-amino-azo-benzene, confirms the conclusion that the vein tip is completely enclosed within an endodermis whose cells are in the granular secondary stage. If a pinna of *Dryopteris* be macerated for about three hours in concentrated sulphuric acid and then gently pressed out on a slide under a cover glass most of the cortical tissue has disappeared and the veins can be seen completely enclosed in a network of Casparian strip, thrown into the usual regular close undulations by the strong acid (Fig. 9).

All the suberin has disappeared from this preparation, but the presence of the suberin can be demonstrated by clearing a pinna in Eau de Javelle, then scraping away the cortical tissue so as to expose the veins and finally boiling the remaining tissues well in Sudan III glycerine. The vein then appears covered to the very tip with masses of suberin globules (Fig. 8 B).

Roots have been occasionally examined during these investigations but nothing can be added to the very full account of the endodermal structure in this organ given by Rumpf.

In the light of the above data as to the structure of the endodermis it is possible to discuss the results of such physiological experiments as have been attempted. Certain details of endodermal structure, such as the thickening of the outer tangential wall in some species, will be discussed in a later section.

THE PHYSIOLOGICAL FUNCTION OF THE ENDODERMIS

As emphasised by Bower (4), the endodermis forms a surface layer separating the vascular system from the cortical ground tissue throughout its length and it is therefore of the first importance to know to what extent water and solutes can be exchanged between the tissues separated by this layer. In the case of water, there is no doubt that its passage, under osmotic control, can take place through the protoplasts of the primary endodermis, and our problem resolves itself into an experimental study of two processes: (i) the passage of water across a secondary endodermis; (ii) the passage of solutes across the endodermis.

(i) *The passage of water across a secondary endodermis.*

This problem has proved very inaccessible to direct experimental attack. Structural considerations discussed later agree with such experimental observations as have been made in suggesting that in the "granular" and cracked stages the secondary endodermis readily

permits the passage of water, but that its diffusion across the continuous suberin lamella in its intermediate firm condition is very much impeded if not entirely prevented.

The fronds of *Dryopteris Filix-mas* permit one method of attack upon this problem, because in the young growing frond the endodermis at the swollen base of the petiole remains primary, in the middle region the endodermis is in the continuous lamella phase, in the upper region it is in the "granular" phase. If now the amount of water in the rachis in these three regions is separately determined by drying to constant weight at 100° C., results of the following type are obtained:

Upper region ...	...	... 80 % water*
Middle region ...	...	... 54 % ,,
Basal region ...	...	... 65 % ,,

* Calculated on the original weight.

If water is previously driven into the frond under mercury pressure the result is only to accentuate these differences. On the other hand, if the rachis of a fully adult and nearly withered frond is taken the following figures are obtained:

Upper region ...	...	... 62 % water
Middle region ...	...	... 63 % ,,
Basal region ...	...	... 62 % ,,

If now water is driven into the frond under pressure, naturally the base shows a rather greater gain, but typical figures obtained in an experiment are:

Upper region ...	...	... 82 % water
Middle region ...	...	... 82 % ,,
Basal region ...	...	... 87 % ,,

These results have frequently been checked by the microscopic examination of sections from the same rachis. In every case when a low original percentage of water is found in the rachis and cannot be materially increased by driving water in under pressure, the endodermis in this region shows the continuous unbroken suberin lamella: if the original water content is high the suberin lamella is in the granular stage, or (as at the base of the rachis) has remained primary: if the original water content is low but can be materially raised by forcing in water under pressure, examination shows the suberin lamella in the cracked final stage.

Experiments were also carried out upon the absorption of water by pieces of rachis (whose surface had been scraped) with the cut ends waxed, and compared with pieces whose ends were unsealed. In every case absorption took place more slowly and to a lower

maximum value in the waxed pieces. In a typical experiment with *Dryopteris Filix-mas* the waxed pieces gained 44 per cent. as against 81 per cent. gain in the case of the unwaxed rachis; in *Dicksonia* sp. the figures were 45 per cent. against 110 per cent. In these cases experiments were made with pieces from the centre of the rachis of the young frond with complete, unbroken suberin lamellæ.

In other cases the loss of water from the ends of pieces of rachis which had been partly dissected out was measured, the cortex of some only being blocked by wax, while of others only the vein ends were blocked. These experiments showed greater loss of water from the cortex, but some loss also from the pieces with the vascular bundles exposed. Pieces of elder twig (without an endodermis) and fern rachis were left to dry side by side in dry air, the cut ends being sealed in both cases. The general result was that the elder twig without endodermis reached a constant weight long before the fern rachis, which, after a first rapid fall, continued to lose weight very slowly for many weeks, presumably as the water slowly migrated from vascular system to cortex.

Two other sets of experiments have a bearing upon this problem:

(1) Pieces of the rachis were cut under water and then sealed at one end, the other being attached to a capillary tube by a piece of rubber tubing filled with water so that the meniscus is at a definite level in the tube. The rachis was then left under water. (Cf. Priestley and Armstead(17) for many experiments of this type with Angiosperm stems.) If the suberin lamella is continuous the water remains at the same level for many days, but if the endodermis is primary or at the granular secondary stage the level of the liquid rises appreciably as water enters the vascular system from the cortex.

(2) From growing fronds of *Nephrodium molle* and of the male fern which had a complete secondary endodermis except at the base of the sori, the sori were removed and the cut surface sealed with wax, control experiments showing that this thin surface of wax cooled so rapidly that it could be applied to such a surface without the tissue withering throughout the thickness of the frond even just below the wax. When these fronds were left growing in the cool greenhouse their leaves dried and withered, being apparently unable to obtain an adequate supply of water from the veins now that the channel through the base of the sorus was interrupted.

To all of these experiments objections can be raised; but taken together with the evidence for the fatty nature of the suberin lamella they seem to show that when the suberin lamella is continuous very

little water passes across it, not enough for the needs of an actively transpiring or a growing tissue. If, on the other hand, the lamella is granular or if it has cracked, as it does with age, then water can pass readily across the endodermis when driven out by a positive pressure in the vascular system or withdrawn by the osmotic action of the surrounding cortical tissues.

(ii) *The passage of solutes across the endodermis.*

The permeability of the endodermis to solutes has been studied in three ways. In one series of experiments the solutions are sucked in at a cut surface as the result of transpiration from the leaves; in another series liquid was driven into the plant by the pressure of a mercury column, precautions being taken to avoid the accumulation of air bubbles at the cut surface. Some experiments were done by this second method upon the movements of sugars into the vascular bundles, but a third method previously employed by Bäsecke throws more light upon this subject.

Solutions at a toxic concentration will naturally penetrate into the primary endodermis and unless a heavy impenetrable precipitate is formed in the cells the toxic solution will pass through the endodermis. Experiments with lead nitrate solutions, the distribution of lead in the plant being subsequently studied by mounting sections in ammonium hydrogen sulphide in glycerine, should throw some light upon the capacity of the secondary endodermis to retain inorganic solutes as the salt readily passes through the primary endodermis. On the other hand, experiments with dyes may throw a little light upon the behaviour of the primary endodermis to organic solutes.

(*a*) *Experiments with lead nitrate solutions.* If lead nitrate solution (3–5 per cent.) is drawn up the young fronds of *Dryopteris Filix-mas*, *Pteris cretica* and *Athyrium Filix-fœmina* by transpiration, the salt passes through the endodermis into the cortex within 12 hours, although after three hours there is no indication of such leakage. The lead salt accumulates very densely around the vein tips at the margin of the fronds, but does not enter the rest of the blade, except occasionally in some of the basal pinnæ.

Lead nitrate is sucked up by the mature fronds of *Dryopteris Filix-mas*, *Nephrodium molle*, *Pteris cretica* and *Dicksonia*, but does not pass out through the endodermis except at the place of attachment of the sorus and at the vein tips, however long the fronds remain in the solution.

When lead nitrate can be sucked up into an old frond of male fern, it escapes through the endodermis both in petiole and pinnæ.

When lead nitrate was driven into the tissues under pressure, in young fronds of *Dryopteris Filix-mas*, *Athyrium Filix-fœmina* and *Pteridium aquilinum*, it had escaped into the cortex throughout the frond, but it failed to escape through the endodermis of the young frond of *Pteris cretica*. In similar pressure experiments with all mature fronds examined, the fronds being examined usually after 12 hours' injection, the lead nitrate is retained within the endodermis, except at the base of the petiole in *Dryopteris* (where there is a primary endodermis), and in all cases at the bases of the sori and the vein tips. In *Polystichum angulare* there is a slight lateral escape in the pinnæ which can be traced to passage cells in the secondary endodermis (see Fig. 10). In the case of *Nephrolepis exaltata* there is no escape along the vein, which is surrounded with a secondary endodermis, until the terminal hydathode is reached: this opens on to the upper surface of the leaf (Fig. 11), and from here the lead salt escapes and diffuses all round the margin of the leaf.

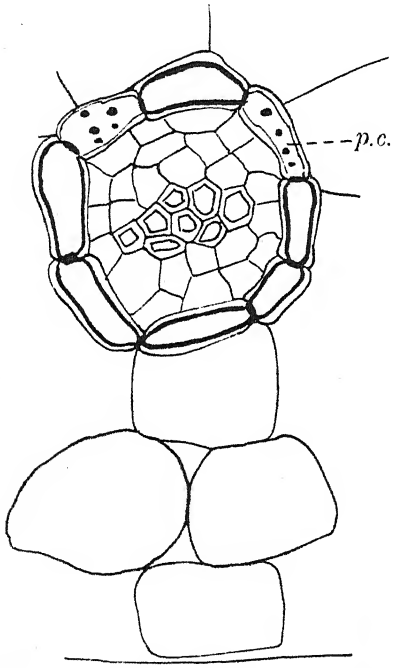


Fig. 10. Transverse section of vein in pinna of *Polystichum angulare*, showing the lateral passage cells, *p.c.*, in the endodermis. (× 750.)

When lead nitrate solution was driven up the frond of *Pteris cretica* for 48 hours, the frond was apparently little affected by this prolonged treatment. On examination no leakage had taken place from the main veins and very slight leakage, if any, from the lateral ones, but the lead salt was flowing freely into the sori through the gap in the marginal bundle and thence had diffused into the leaf tissue nearly up to the midrib of the pinna.

Lead nitrate driven up the old and withering fronds leaks freely into the cortex through the cracked suberin lamella, and with the experience gained in this type of experiment it can now be said with confidence, that after the continuous suberin lamella has once been laid down in the cells of the endodermis, the subsequent transit of lead nitrate across the endodermis can always be traced either to passage cells or to a collapse of the lamella, either due to age or very occasionally to the collapse of a weak lamella under the continuous pressure of the liquid in the veins. On the other hand, the experi-

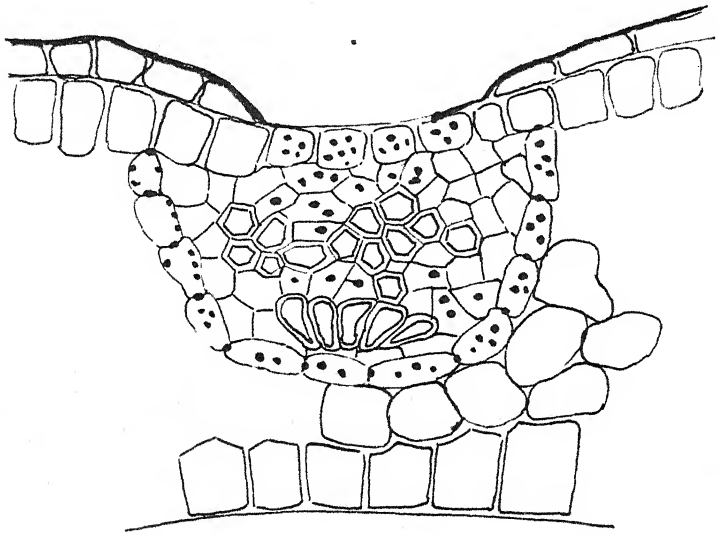


Fig 11. Transverse section of the hydathode of *Nephrolepis exaltata* (× 800). Casparian strip present, together with globules of fat in the endodermal cells; no cuticle present over the centre of the hydathode.

ments with young fronds show that lead nitrate passes readily through the granular stage of the secondary endodermis when driven into the vascular system under pressure, and passes out slowly when the salt is sucked up by transpiration.

(*b*) *Experiments with dyes* have usually been carried out with 0·1 per cent. solution of acid dyes. Basic dyes will usually not pass up the vascular system when under pressure, the frond of *Dicksonia* being the one exception noted to this rule. Here methylene blue passes up readily, but on examination it appears to be mainly in the walls of the phloem, which appear to contain considerable quanti-

ties of pectin. The experiments with acid dyes do not require detailed description. With the secondary endodermis they confirm completely the lead nitrate experiments: the dyes leak readily through the cracked endodermis in the old dry leaf, they are completely retained within the continuous unbroken lamella and they pass slowly and with more difficulty than lead nitrate through the endodermis in the granular stage.

In our experiments, possibly because of the strength of the dye solution used, the dyes usually passed out through the protoplasts of the primary endodermis, but in the case of a hydathode such as that of *Pteridium aquilinum*, which is completely enclosed within a primary endodermis, there is no difficulty in understanding Spanjer's(23) experimental results, in which, when eosin solution was forced into the leaves, water alone emerged through the hydathodes, the eosin being retained. These results are in accordance with many of our own experiments, but with longer duration of experiment or stronger solution Spanjer would certainly have found eosin ultimately escaping from the hydathode through the dead protoplasts of the primary endodermis.

(*c*) *The passage of sugar through the endodermis.* If lead nitrate cannot pass the secondary endodermis with continuous suberin lamellæ, sugar is not very likely to do so, but in view of the importance of the question, 5 per cent. glucose solution was driven up the vascular strand under pressure and the distribution of the sugar subsequently studied by low power microscopic examination after heating in Fehling's solution.

Sugar solution could only be driven up the vascular strand of young fronds with great difficulty, but when this was successfully done it appeared to leak out into the cortex through the "granular" secondary endodermis. No leak through the continuous lamellæ of a more mature frond could be traced.

Observation upon the behaviour of sections from comparable fronds, boiled in Fehling's solution without previous injection of sugar, certainly indicated that the rough test of leakage thus applied had given quite definite results.

But the work of Bäsecke had already placed the conclusion to which these experiments point upon a very secure basis. In partially shaded fronds of *Scolopendrium* he showed that starch completely disappeared from the shaded region except at the base of the sori. In this region it appeared to be retained because the carbohydrates formed in the lighted leaf area continued to diffuse into the vascular

strand through the gaps in the secondary endodermis in this region.

In a first attempt to repeat this experiment, the partially shaded leaves of *Nephrodium molle*, after 16 days' shading, showed only starch at the vein tips, but on anatomical examination it was found that the endodermis under the young sori was practically completely secondary across their bases, probably because the leaves were grown in a cold greenhouse in winter. Experiments repeated in the spring completely confirmed Bäsecke. In the mature fronds of *Athyrium Filix-fœmina* and *Nephrodium molle*, the starch disappears last from the base of the sorus and in a young almost undeveloped frond of *Dryopteris Filix-mas* in which the endodermis is in the granular stage, the starch disappears uniformly from all shaded areas of the leaf. In *Pteris cretica* the disappearance of the starch occurs more uniformly but it goes last from the leaf margin. The starch does not disappear from the guard cells of the stomata.

It would therefore appear that there is a stage between primary and "continuous" secondary endodermis, the "granular" stage, in which the protoplasts of the endodermis are relatively permeable to sugar and the suberin lamella is too unformed to prevent its passage. This point may have some significance in reference to the utilisation of the starch reserve from the ground tissue of such a rhizome as that of *Pteridium aquilinum*.

In a previous paper (18), the question has been raised as to whether the food reserve is lost to the plant. In the last few years experiment has demonstrated its utilisation. In the autumn pieces of rhizome of *Pteridium* were cut out, sections from the ends retained to show the amount of starch present, the cut ends then waxed and the pieces left in soil in the greenhouse until spring. If these short lengths of rhizome contained an apical growing point then in the spring an obvious diminution of starch took place, but if they bore the young stage of a lateral frond the disappearance of starch was usually absolutely complete. In some experiments, rhizomes packed with starch and planted in the spring were completely cleared of starch within a month; possibly the short length of rhizome used in the experiment (1–2 inches) was partly responsible for the completeness of its disappearance. When the disappearance of the starch was noted sugars were looked for microchemically by the method of Mangham (12), but no reducing sugars, forming osazones, were present. On the other hand, on many occasions the osazone reaction has shown the region just below either leaf or stem meristem to be very rich in reducing

sugars, giving both dextrosazone crystals and crystals of different form. These rhizomes, as also part of the rhizome and leaf base of *Dryopteris Filix-mas*, were digested with water and chloroform and the extract tested with starch solution for diastase.

The following results were obtained:

Plants	Action on starch	Formation of sugar
Leaf base and rhizome of *Dryopteris Filix-mas*	Iodine reaction goes immediately at room temperature	Slight reduction of Fehling
Rhizome of *Pteridium* which has lost its starch	Iodine reaction goes almost immediately at room temperature	,,
Rhizome of *Pteridium* which has not lost its starch	Iodine reaction disappears very slowly indeed	No reduction of Fehling

It therefore seems that when the roots begin to drive water into the vascular system in spring, provided there is a region where this vascular system is not separated from the cortex by a continuous suberin lamella, hydrolysis of the starch takes place, the sugar then probably travelling to the growing region by diffusion through the ground tissue. In no case did any starch disappear from pieces of rhizome which bore neither an apical bud nor young fern frond.

THE ENDODERMIS AS A PROBLEM OF CAUSAL ANATOMY

In a succeeding section the attempt is made to show that the progressive development of the endodermis exerts a profound effect upon the development of the cortical tissues, which are dependent for their supplies of sap from the vascular system upon facilities of transit across the endodermis. To make full use of this conclusion in the interpretation of the structure of the fern it is obviously necessary to obtain knowledge of the factors in development which determine the successive appearance of the endodermal stages. This point has therefore been kept constantly in mind and a number of experiments directed to its elucidation, but it must be confessed that the whole problem of the causal anatomy of this layer is still very obscure and all that is attempted now is to draw attention to certain factors which seem to be constantly associated with the two important structural stages—primary and secondary.

(i) *The primary endodermis.* Certain grounds can be given for the view that the formation of a primary endodermis involves at least the coincidence of three different factors: (1) the presence of unsaturated fatty acids, derived originally from the phloem; (2) the young, almost meristematic condition of the wall of the endodermal cell; (3) the

presence of air. Reference to earlier papers (15, 18) will show that the same conditions seem to be at work in the differentiation of the primary endodermis of the Angiosperm; and some further studies, as yet unpublished, have reinforced this conclusion.

Staining with osmic acid shows that in the region of vascular differentiation the young bundles of the *Pteridium* rhizome and frond are packed with strongly reducing substances, the cortex being comparatively free.

Unsaturated substances appear to be prevalent in the meristematic tissue itself, but in the ground tissue differentiation is associated with the appearance of air spaces, and after that time very little reduction of osmic acid takes place in this tissue, the last layer of tissue to stain deep black being the developing endodermis.

As the development of the bundle proceeds, the fat reaction and the strong reduction of osmic acid, so characteristic of the xylem parenchyma and the phloem, gradually become less marked, the formation of the Casparian strip representing a stage where there is an appreciable reduction in the osmic reaction of the internal vascular tissue.

Judging from the very strong acid reaction of the Casparian strip this fatty acid must be attached to the wall by its unsaturated HC = CH linkages. In the case of the Angiosperm further evidence for this was obtainable by the capacity of ethylene (a common impurity in coal-gas) to prevent the formation of the strip. Fronds of *Pteridium* grown in coal-gas have not supplied any evidence of this nature, the strip being formed apparently quite normally, but in view of the acid reactions of the strip the attachment of the fatty acid to the wall cannot be by the carboxyl (—COOH) group, whilst the resistance the strip offers to strong alkalis suggests that the chemical combination of the fatty acid has been followed by its oxidation and condensation, so that it is no longer readily hydrolysed by alkali.

The Casparian strip always forms at a very early stage in the development of the bundle: so far no exceptions to this rule have been found. It appears, as in the case of the gap in the base of the sorus of *Pteris cretica*, that the wall at a later stage of differentiation has not the same power of retaining the fatty acid by chemical leakage, possibly because some constituent of the meristematic wall has disappeared during differentiation (Priestley and Tupper-Carey (19)).

The whole wall of the developing endodermal cell probably has this affinity for the unsaturated acid, but the acid is only per-

manently retained in the radial and transverse walls because in these positions the inward diffusion of oxygen from the adjacent intercellular spaces produces, from a mobile, liquid, partly soluble acid, a solid, varnish-like, insoluble substance which when once formed prevents the outward diffusion of the acid on to the outer tangential wall.

The above factors can then be put forward as contributory to the formation of the Casparian strip in the Filicineæ. Unfortunately, in the present state of our knowledge, they do not provide a solution to such problems as the reason for the absence of the strip in the base of the sori of *Pteris cretica* or the total absence of the primary endodermis in the fronds of the Marattiaceæ. In this latter case it is perhaps worth noting that the source of the fatty acids of the strip appears to be the phloem, and Rumpf's observations show the endodermis in the root appearing first opposite the phloem, whilst Brebner (5) draws attention to the fact that the Marattiaceæ have, as a general peculiarity, the first formed phloem elements of the leaf steles in an end-arch position.

(ii) *The secondary endodermis.* In the Marattiaceæ and Ophioglossaceæ the endodermis, if present, is primary in the adult tissue. With the exceptions of the "storage rhizomes" and *Trichomanes* it is secondary throughout the Filicineæ. At present it is impossible to analyse the developmental conditions with any confidence and to ascribe the appearance of the suberin lamella to definite features, but the following points seem to be relevant.

If no secondary lamella forms, then the primary endodermis in the adult tissue is a very permeable boundary layer between cortex and stele, because the living protoplasts of the endodermal cells grow more permeable with age. The secondary endodermis, on the other hand, represents an endodermal layer in which the increasing permeability of the protoplast has been associated with the accumulation of oil or fat at its surface. Sometimes these oils or fats have apparently come from the interior of the protoplast, because in these cases the suberin lamella is continuous all round the inside of the cells, is equally thick on all sides, and is deposited throughout simultaneously.

In other cases, while some constituents of the layer may be proceeding to the surface from the interior of the protoplast as its permeability increases, another constituent is reaching it from the stele within. As this substance cannot diffuse out through the Casparian strip, the result is virtually a precipitation membrane, which

is produced over the inside wall only of the protoplast, extending always from the margin of the strip.

The possibility of precipitation is also indicated by the relative neutrality of the suberin lamella, which is particularly evident when the lamella is first being deposited, and is indicated by its pink reaction at this stage with Nile blue sulphate (Bolles Lee (3), *loc. cit.* p. 362), and its yellow reaction with di-methyl-amino-azo-benzene and with methyl red.

These neutral reactions suggest the possibility of ester formation between fatty acid and some relatively basic constituent, probably containing reactive hydroxyl groups. (Phenols are obviously not excluded, and it is worth noting that in the developing stages both the xylem parenchyma and endodermal cells give dense tannin reaction with the vapour of amyl nitrite, and in *Dicksonia* the red lignin reaction, obtained with hydrochloric acid alone, indicates the presence of phloroglucinol.)

It is a remarkable fact that at the time these globules of oil or granules of fat are coalescing into a lamella at the surface of the stele the vascular contents of the stele must often contain liquid under considerable pressure, as the young cut fronds will force liquid into attached tubes at a rapid rate (1·5 c.c. of liquid per hour has been collected from quite slender fronds). No further hint has yet been obtained as to the conditions which favour this suberin lamella formation and which at the same time will explain its absence from the storage rhizomes and petiolar bases of such a form as *Dryopteris Filix-mas*.

The Endodermis as a Factor in Causal Anatomy

It is now proposed to examine the effect upon the general structure of the fern of the presence of an endodermis around the vascular tissue throughout root, stem and leaf from the time of its first development.

This early and complete development of the endodermis is characteristic of the Filicineæ. In Angiosperms it is at least very rare, whilst in other groups of the Pteridophyta there are numerous exceptions to its occurrence (Mager (11)), and there is no group other than the Filicineæ in which a secondary endodermis is such a constant feature.

In a previous series of papers (16–21) the importance of the presence of a functional endodermis has been stressed. A primary endodermis when the protoplasts are relatively impermeable and a secondary

endodermis so long as the suberin lamella is impermeable, will certainly restrain the free nourishment of meristematic tissues or potentially meristematic tissues which are separated by such a layer from the vascular tissues. This is inevitable because the meristem requires for its continued growth a constant supply of organic solutes in the sap reaching it. Such solutes usually arrive from the vascular cylinder and will certainly not pass through a suberin lamella and probably not through a healthy young protoplast. But the typical process of development in the fern shows the vascular cylinder separated from the tissues external to it by just such a young primary or continuous secondary endodermis throughout the greater part of its development. One may anticipate, therefore, that the meristematic tissue will mainly be confined to apical regions, and this is the characteristic feature of Filicinean growth in root, stem and frond. In the stem it is striking that not only is branching confined to the meristem at the apex, but the origin of lateral roots is limited to the region close behind the apex, practically to the region where the endodermis is primary. This may be associated with two facts—in the first place the root initials are endodermal cells, and these will certainly lose all meristematic properties as they pass out of the primary stage, in the second a root arising later than this would have to make its way out through a very resistant cortex, the ground tissues rapidly toughening as they dry after the deposition of the suberin lamella. So far as it has yet proved possible to study them, the dormant lateral axes found upon the leaf bases in *Dryopteris Filix-mas*, *Pteridium*, etc. seem to be associated with a permanent gap in the secondary endodermis. This is particularly marked in the male fern, in which type it is possible to propagate the plant from the base of the separated leaf: the permanent primary endodermis in this region has been referred to frequently, and with the passage of time the protoplasts of this primary endodermis probably become very permeable.

So far as they are known to us, the regions of continued meristematic growth found in the Filicineæ may all be associated with gaps left in the endodermis or with regions enclosed by a primary endodermis: a typical case is the fairly frequent production of a vegetative bud from the region of an abortive sorus. On the other hand, it is only upon the roots of Ophioglossaceæ or the petiole and stipules of *Marattia*, *Angiopteris*, etc., *i.e.* in organs possessing a primary endodermis persisting as such for many years, as in *Ophioglossum* root, or without an endodermis at all, that parenchymatous tissues

of almost any cortical region may be stimulated to adventitious meristem formation.

Exactly the same argument applies to the appearance of such a tissue as a cork phellogen. Of frequent occurrence in the leaf base of the Marattiaceæ, such tissues are practically unknown, even in response to wounds, in the Filicineæ (Holden (8), Priestley and Woffenden (20)).

In the Angiosperm a functional endodermis may frequently be found in the stem but is rare in the petiole and almost unknown in the lamina. With this fact may undoubtedly be correlated the basal growth of the leaves of Monocotyledons and the petioles of Dicotyledons, together with the general long-continued meristematic activity in the lamina, especially near the margin, in Dicotyledons. On the other hand, the Fern frond, with its endodermis keeping pace with its vascular development, naturally has the supply of sap directed towards the apical meristem, and growth throughout is apical, the terminal pinnæ in a type like *Adiantum* being almost comparable to the deltas formed at the discharging mouth of a river system, the serrated margin of other pinnæ showing an obvious relation to the termini of the numerous lateral veins and the absence of a connecting marginal vein (see Fig. 6, *Pteris cretica*, p. 171).

When once the leaf blade has been spread out to the light, its subsequent photosynthetic and transpiring activity requires examination in the light of the fact that diffusion and osmotic gradients will certainly be established throughout the lamina and will centre around the only possible routes of free ingress and egress—the bases of the sori and the tips of the veins. As a rule in the ferns there is no definite absciss layer at the leaf base. Bäsecke cites exceptions, but unfortunately we have been unable to examine these types to see if there is any correlation between its occurrence and the retention of a primary endodermis in the leaf base. The general rule in ferns appears to be that the suberin lamellæ gradually form continuously over the few remaining gaps within the endodermis, following probably upon the cessation of sap flow under pressure as the leaf development becomes commensurate with the root system, and after that the mesophyll, thus isolated from the vascular system, slowly dries and withers.

The transition from the primary endodermis to the secondary type has also an important reaction upon the structure of the cortex. If the suberin lamella fails to form as the endodermis grows older solutes undoubtedly pass freely between vascular system and ground

tissue, whilst with the formation of a suberin lamella such exchange is practically prevented. It is in this sense that we would interpret the difference in form and structure between the bulky, juicy, rhizome of the male fern, with its tissues soft and white in section, and the hard, dry tissues of the older region of the rhizome of the bracken, relatively brown in sections. In view of our ignorance of the causal factors in the production of a suberin lamella, we are left with the problem of the causal anatomy of these distinct types of rhizome incompletely solved, but it seems more correct at present to consider the absence of the suberin lamella as responsible for the bulky, food packed rhizome than the presence of these food supplies as responsible for the absence of the lamella.

A similar correlation undoubtedly holds between the fleshy white roots of Ophioglossaceæ and Marattiaceæ with their permanent primary endodermis and the thin fibrous brown roots of the Leptosporangiate Filicineæ with their early development of a secondary endodermis. A parallel case amongst Angiosperms seems to be supplied by the types of fleshy white and fibrous brown roots found in the same plants of Wheat and Barley, the anatomy of which has recently been described by Brenchley and Jackson (6, 9), whose figures suggest that the same difference in endodermal structure can be found between the two different types of root in these plants also.

The brown colouring substance, the "vagin" of Bäsecke, can be readily removed by soaking sections in Eau de Javelle, which thus becomes a most valuable reagent in the study of the fern. "Vagin" appears to be a humin-like derivative from carbohydrate, produced by chemical condensations and oxidations occurring in these substances as the result of the dehydrating conditions which follow upon their isolation from the vascular strands by a suberin lamella. A considerable amount of the substance can be isolated from the tissue by extraction with alkali and subsequent precipitation with acids; on fusion with potash the presence of constituents containing a benzene ring is shown by the identification of catechol or protocatechuic acid amongst the decomposition products (Priestley and North (18) and literature there cited). In some of the ferns examined, as *Dryopteris Filix-mas* and *Polystichum angulare*, the outer tangential wall of the endodermis is greatly thickened and coloured almost black by deposition of this "vagin": after its removal by Eau de Javelle, the thickened wall gives clear cellulose reactions and the structure of the endodermal cells within can be made out (Text-figs. 3 and 5). The thickening of the walls of the ground tissue appears to be of two

distinct types, the distribution of these within the root system having been fully described by Rumpf. In one type, the walls of a single cell are unevenly thickened as in the case of the outer tangential wall of the endodermis just described, and as in the sclerenchyma of the rhizome and frond of *Pteridium*, in which at the margin of the tissue the walls gradually taper down to normal thickness. The same type is described by Rumpf as occurring opposite the protoxylem groups in the roots of *Dryopteris Filix-mas*. In this type of tissue the material for formation of the wall appears to be diffusing along the walls between the protoplasts and to accumulate unequally within them, probably owing to the drying effect produced by exposure to the air at contact with intercellular spaces. The result is that if a small patch of such tissue is examined and its thickness considered, its centre of formation will appear either to be an intercellular air space, as in the sclerenchyma of *Pteridium*, or a vascular sap supply, as in the outer tangential wall of the endodermis or in the brown sclerenchyma of the roots. The browning of this sclerenchyma, which characteristically takes place, has also been described by Bäsecke, and appears to progress slowly inwards from the outer surface of the plant or to work outwards around an intercellular air space.

This type of sclerenchyma, which is present in the outer layer of the rhizome and frond of practically all Filicineæ in the region where there is a completed secondary endodermis, appears therefore to owe its production to the accumulation of substances upon the wall and their drying and subsequent oxidation after isolation from the vascular system. It appears to be quite different in character from the tissue in the Angiosperm described as sclerenchyma; but since its centre of formation is often indicated as an intercellular air space it shows greater similarities to collenchyma, from which of course it differs entirely in the nature of the constituents of the wall.

The other type of sclerenchyma, which is fully described by Rumpf, lies opposite to the phloem group in the cortex of many roots and undoubtedly shows greater resemblance to the sclerenchyma of the Angiosperm stem, although the latter usually forms *within* the endodermis or starch sheath. In this tissue the walls are thickened evenly around the cell, evidently being deposited by the protoplast uniformly over its whole surface: since, however, from the details of development given by Rumpf, it always forms after the vascular system is isolated from the cortex by a secondary endodermis, the activity of the protoplast appears to be determined by the tendency towards

chemical condensation brought about by the loss of the vascular sap supply. The frequent restriction of this tissue to regions opposite the phloem strands would suggest that the cortical cells in this region alone have been able to accumulate the necessary carbohydrate supplies before the completion of the suberin lamella cut off the supply and led to their condensation upon the wall. These walls undergo less subsequent change and are not so brown with infiltrated vagin as the other type of thickened wall, which is more usually found opposite the xylem.

In conclusion, a few words may be said about the cuticle in the Filicineæ. This is described by de Bary ((1), *loc. cit.* p. 81) as a brown structure frequently involving inner as well as superficial walls, or

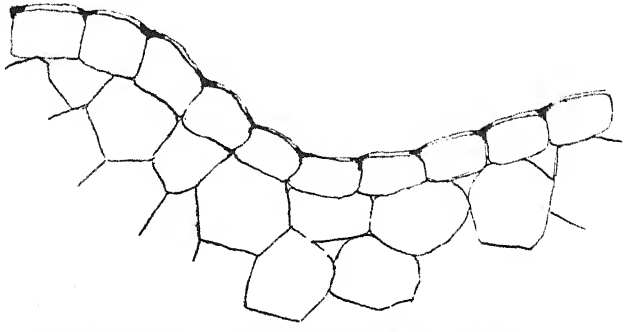


Fig. 12. Section of epidermis of frond of *Polystichum angulare*, the fat-impregnated regions of the outer wall are shown black. (× 800.)

even the walls of inner layers of cells. But the description applies to the "vagin"-impregnated wall of the outer cells of the ground tissue and not to a superficial fat deposit homologous with the cuticle of the Angiosperm.

Such fat deposits are, indeed, scanty throughout the Filicineæ, and the reason seems to be that the main source of the normal Angiosperm cuticle appears to be the fat supply of the vascular system (the ground for this statement rests in part upon some work by Miss Beatrice Lee in this Department, *Annals of Botany*, **38**, 1924), and in the group now under consideration these fats are largely retained within the endodermis, where they accumulate, especially as the suberin lamella. In the rhizome of the adult plant no trace of cuticle can be found, and in the frond, on staining with Sudan III, it will frequently be found confined almost entirely to the regions opposite the vertical walls of the epidermal cells, as if it had accumulated there after diffusion to the surface along these walls (Fig. 12).

On the other hand, in the Marattiaceæ, where the fat is not retained within the endodermis, quite a thick cuticle will be found upon the frond.

Summary

1. In the Filicineæ every vascular strand is enclosed within an endodermis throughout its whole length from its earliest differentiation.

2. This endodermis is at first primary in structure, having the Casparian strip alone; it afterwards usually passes into the secondary stage with suberin lamella.

3. The Casparian strip is regarded as a deposit of fatty acid nature in the substance of the original, little differentiated wall.

4. The suberin lamella may either form a continuous layer covering the whole inner surface of the original wall, or it may spread over the inner tangential wall only, from the region of the Casparian strip.

5. The suberin lamella consists of fatty substance, but is not acid in reaction, and it is not deposited on a basal cellulose-like substance as in the Angiosperm. Its development can be traced through three phases, (i) globular or granular fatty deposit, (ii) a continuous solid lamella, (iii) a cracked and disorganised lamella.

6. The presence of these phases and the absence of a tertiary endodermal stage, both features distinguishing the Fern endodermis from the Angiosperm endodermis, can be correlated with the absence of the deposition of the basal cellulose-like substance found in the Angiosperm.

7. The primary endodermis allows the exchange of water and inorganic solutes between vascular and ground tissue, but probably impedes the outward diffusion of organic solutes whilst the protoplasts are young and relatively impermeable.

8. The suberin lamella allows exchange of water and solutes in phases (i) and (iii), (para. 5), but practically prevents all exchange in phase (ii). So far as is possible an experimental basis is supplied for these statements as to the function of the two endodermal stages.

9. The problem of the origin of the two types of endodermis is discussed, but although certain tentative conclusions are possible no reason can be suggested, (*a*) for the absence of an endodermis in certain groups of ferns, (*b*) for the failure to reach the secondary stage in certain regions, as in the "storage" rhizomes.

10. The constant presence of the endodermis seems to throw light upon certain characteristic features of Filicinean structure and development. From this standpoint are discussed:

(*a*) The constant apical development normal to root, stem and leaf;

(*b*) The position of adventitious buds;

(*c*) The exceptional occurrence of phellogen;

(*d*) The origin of lateral roots;

(*e*) The development of the frond, and its physiological functioning.

11. The presence of a secondary endodermis shows interesting correlation with

(*a*) The production of a dry, thick-walled "vagin"-impregnated cortex. In this connection two distinct types of sclerenchyma in the Filicineæ are distinguished.

(*b*) The slight production of a fat-containing cuticle such as is characteristic of the Angiosperm.

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THE DIFFUSION OF IONS FROM LIVING PLANT TISSUES IN RELATION TO PROTEIN ISO-ELECTRIC POINTS

BY W. H. PEARSALL AND J. EWING

(With 1 figure in the text)

IN dealing with living or dried plant tissues in solutions of varying hydrogen-ion concentration, our attention was drawn to the fact that the behaviour of the tissue was, in some respects, largely determined by the hydrogen-ion concentration of the external solution. There appeared to be a relation between the facts observed and the iso-electric points of the tissue proteins. It is the object of the present communication to record some of the experimental observations which support this assumption.

Although the iso-electric point of a protein extracted from plant tissue can usually be determined without great difficulty, it does not necessarily follow that the behaviour of the protein *in vitro* is any indication of its behaviour in the protoplasmic complex. The establishment of any relation between protein behaviour *in vitro* and the behaviour of the protoplasmic membrane, seemed therefore to be a matter of great importance.

Lastly, in previous investigations(7) on the iso-electric points of tissue proteins, we have repeatedly been faced with the difficulty of extracting any appreciable quantities of protein from active

tissues, and we wished, therefore, to explore methods which seemed to render it possible to determine protoplasmic iso-electric points in cases where direct determinations on extracted proteins were not possible. The methods employed in the following experiments seem to offer distinct possibilities from this point of view.

METHODS

The basis of the experiments was the comparison of the rates of diffusion (of some easily determined ion) from living tissues kept in solutions of different hydrogen-ion concentrations; and the chloride ion seemed convenient for this purpose.

As a large number of comparable estimations had to be done in a short time, rapidity of estimation was an important factor in deciding upon the method to be used. Equal volumes of the tissues to be examined, were placed in a series of conical flasks containing equal volumes of solution. The chlorine in this solution was estimated by the silver nitrate method, potassium chromate being used as indicator, and the titrations were carried out to a definite colour standard as end point. The acid series of solutions were neutralised with dilute sodium hydrate before the chlorine was estimated and three chlorine estimations on 20 c.c. of liquid were carried out on each solution. The actual gain or loss of chlorine in the solution was then determined by comparison with a blank series of solutions containing no tissue.

The tissue used, potato, was cut into cylinders with a cork borer, each cylinder being 3·8 cms. (1·5 inches) long and 0·8 cm. in diameter, and three cylinders were put into each flask. This method was adopted, as in order to get the required range of hydrogen-ion concentration, at least ten solutions were required, and each of these had to be put up in triplicate (at least) so that time effects could be observed. Thus thirty equal masses of tissue were required for such a series of estimations. Weighing each of these proved impracticable, so they were prepared as above. The volume of the tissue used in each flask, as determined separately on twenty samples of three pieces each, was $6{\cdot}6 \pm 0{\cdot}3$ c.c. These were dried with filter paper and the average fresh weight of three pieces was $6{\cdot}75 \pm 0{\cdot}27$ gms. The hydrogen-ion concentration was determined colorimetrically unless otherwise stated (Clarke (1)) in both the solutions and also in the sap of the potato. In the latter case, there may be an error due to the turbidity and protein content of the sap. We only required these figures for comparison, however, to see if the *p*H value of the

sap underwent any striking change. The sap reaction was determined from the liquid obtained when potato cylinders were crushed up with 1 c.c. of distilled water. The reactions thus obtained were identical with those made on sap squeezed from larger quantities of potato under pressure.

Potato juice

The expressed juice of the potatoes used had normally a reaction of *p*H 5·4 to 5·6. If, however, the suspended matter was removed by filtration through animal charcoal under a pressure of 0·7 atmosphere, the sap had a reaction of *p*H 6·2. Such treatment makes the juice quite colourless and removes all but traces of protein. The chlorine content of the filtered sap was between 1·0 and 0·96 gm. per litre[1], or equal to about $N/36$ sodium chloride. This is certainly a minimal value for the free chlorine of the sap, since some may have been lost with the suspended matter in filtration, and moreover, the *expressed* sap of a tissue is usually more dilute than the actual sap (Dixon and Atkins(4)). Hoagland and Davis(5) found that this applied to the chlorine content of *Nitella* sap. This minimal value can be checked by another method. The loss of chlorine from 6·6 c.c. of the same potato observed on killing the tissue, was 5·15 mgms. after three days, and values close to this were frequently obtained in our experiments. The potato used contained about 78 per cent. of water by weight, *i.e.* 5·15 c.c. in 6·6 c.c. of tissue. Since on this basis, 5·15 mgms. of chlorine might be lost from 5·15 c.c. of sap, we arrived at the same figure, about 1 gm. per litre, for the chlorine content of potato sap.

The external solutions used contained at most a total concentration of 8 c.c. of tenth normal chloride in 100 c.c. of solution, so that the chloride concentration of the solution did not exceed $N/125$. The chloride concentration of the sap was thus always much higher than that of the external solution, and, in the majority of cases, chlorine-ions diffused from the potato into the external solution. In solutions of pure sodium chloride, no significant difference in the rate of diffusion was observed by us, if the external solution varied between $N/500$ and $N/125$. The potato lost to such solutions 0·15 to 0·25 mgm.

[1] These figures refer to the variety of potato used in these experiments. The chlorine content of the expressed sap from different varieties of potato varies between 0·8 and 1·7 gm. per litre. Gravimetric estimations on either the filtered sap or on the ash obtained from this sap (neutralised with alkali) give the same values for chlorine as the volumetric estimations. Gravimetric estimations also show that the unfiltered sap may contain from 15 to 20 per cent. more chlorine, this being lost during filtration through animal charcoal. The values obtained indicate that there is no organic chlorine, it is all in the ionic state.

of chlorine in 12 hours, the differences being apparently unrelated to the external concentration. The loss of chlorine in distilled water was much larger, about 0·7 to 0·85 mgm. in 12 hours.

As the experiments were to be made on living tissue giving off carbon dioxide the distilled water used in these experiments had always been exposed to the atmosphere and was saturated with carbon dioxide at the existing temperature and pressure. Hence its *p*H value was low, usually *p*H 5·3–5·5.

In the following tables the results are calculated as chlorine lost from 10 gms. of tissue, this being a more convenient unit than 6·75 gms., the weight actually used—and the method of expression only involves a proportionate increase of all results.

Relation of Chlorine Movements to Hydrogen-ion Concentration

The simplest system that can be examined is one in which the tissue is exposed to the action of various concentrations of hydrochloric acid. This has obvious disadvantages, particularly that the chlorine content of the external solution varies with the hydrogen-ion concentration, but it is a convenient and simple starting-point. Tables I and II contain the results of experiments dealing with this system, and these results are expressed graphically in Fig. 1. Considering first the results after 4 hours, we find that most of the tissues show an initial loss of chlorine; but a few show a gain, viz. those between *p*H 4·5 and 5·2 at 4 hours. The loss of chlorine by the tissue in solutions of higher hydrogen-ion concentration than *p*H 4·5 is probably to be attributed to the injurious effect of the hydrogen-ion concentration. Nos. 3 and 4 in Table II point to the critical *p*H value below which this loss takes place as lying at about *p*H 4·30.

Table I. Movements of Chlorine to or from 10 gms. of potato in Hydrochloric Acid

100 c.c. of solution containing *N*/100 HCl		4 hours		18 hours		32 hours		50 hours	
c.c.	*p*H	*p*H	Chlorine gained or lost by tissue	*p*H	Chlorine gained or lost	*p*H	Chlorine gained or lost	*p*H	Chlorine gained or lost
12·0	3·1	3·3	−2·20	3·6	−5·80	4·0	−6·00	4·2	−8·80
0·9	4·7	4·9	+1·28	5·5	+1·04	6·4	+0·84	6·6	+0·05
0·6	4·8	5·0	+0·32	6·2	+0·56	6·5	−0·08	6·7	−0·88
0·3	5·1	5·5	−0·68	6·4	−1·80	6·5	−2·48	6·7	−2·80
0·0	5·4	5·7	−1·12	6·6	−2·00	6·6	−2·68	6·8	−3·40

The *p*H value of the sap remained constant at *p*H 5·6 in the above series, except in No. 1, where the readings at 4, 18, 32 and 50 hours were respectively 5·4, 5·0, 4·8 and 4·7.

TABLE II. Movements of Chlorine to or from 10 gms. of potato

100 c.c. of solution containing $N/100$ HCl		4 hours		19 hours		32 hours		50 hours	
c.c.	*p*H	*p*H	Chlorine gained or lost	*p*H	Chlorine gained or lost	*p*H	Chlorine gained or lost	*p*H	Chlorine gained or lost
30·0	2·6	2·8	−2·04	3·2	−4·08	3·6	−4·64	3·8	−8·00
7·5	3·4	3·6	−2·24	4·5	−2·00	5·6	−2·60	6·5	−4·00
3·0	3·9	4·5	−0·16	5·8	−1·44	6·2	−1·60	6·6	−1·60
1·2	4·4	4·7	+1·20	6·2	+0·80	6·4	0·0	6·8	−0·40
0·0	5·4	—	—	—	—	—	—	7·0	−4·00

The internal *p*H only changed in No. 1 where the four successive readings were 5·3, 5·2, 4·9, 4·4. The normal tissue was 5·6.

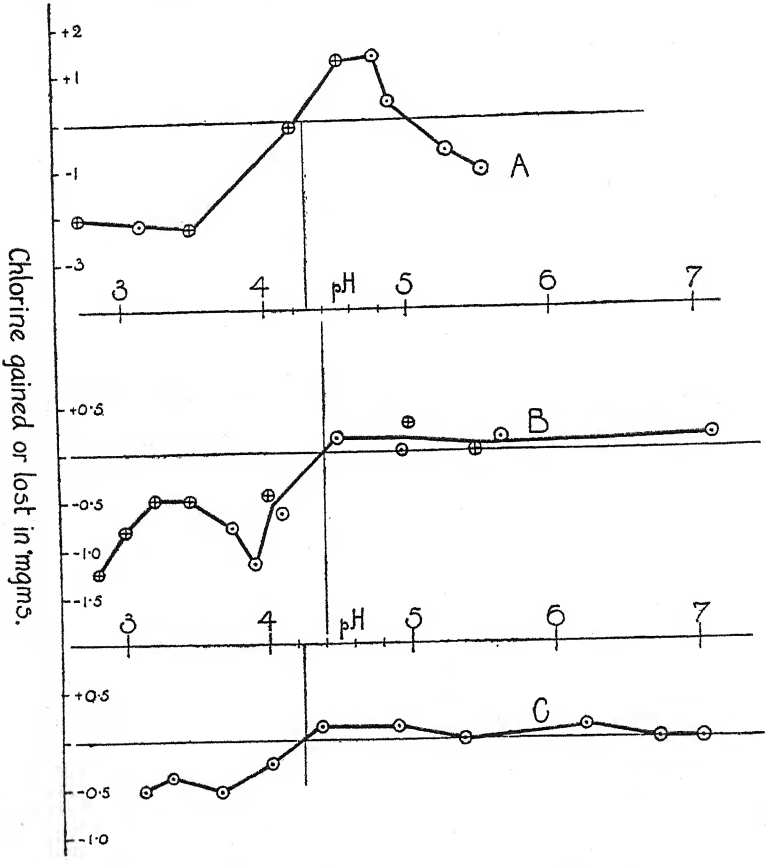

Fig. 1. Gain or loss of chlorine from plant tissue at various hydrogen-ion concentrations. A—Potato in hydrochloric acid solutions; B—Potato in sodium chloride and hydrochloric acid solutions; C—Carrot in similar solutions. In B and C the figures are given as gains or losses compared with loss in pure salt solution.

The average *p*H value at which the tissue lost the given quantities of chlorine may be assumed to be roughly the mean of the initial *p*H and that at 4 hours. For Nos. 3 and 4 (Table II) the values would be respectively *p*H 4·2 and 4·55, and from the graph this gives no loss at about *p*H 4·30. Below this *p*H value, all the solutions cause a heavy loss of chlorine from the tissue.

In the least acid solutions there may be also a loss of chlorine, but it is probable that this is due to a different cause, the negligible amounts of chlorine in the external solution. It has already been shown that potato sap contains a fairly high concentration of chlorine, which diffuses out when the tissue is kept in distilled water. The figures given in Table I show that the amounts diffusing from the tissue increase as the external concentration of chloride falls. If then the external concentrations of chlorine were identical at the various *p*H values, no such loss of chlorine should take place. This is shown by the results in the later tables. It is clear, therefore, that the loss of chlorine from potato in very dilute solutions of hydrochloric acid is not connected with their hydrogen-ion concentration. On the other hand, in stronger solutions, the higher hydrogen concentration causes loss of chlorine from potato through its effect on the protoplasmic membrane. This effect seems to start about *p*H 4·3. Besides this relatively rapid effect of hydrogen-ions upon the tissues, there is a secondary effect, which needs explanation. In Nos. 2 and 3, Table I, and No. 4, Table II, there is initially an absorption of chlorine from the solutions. The amount absorbed gradually falls off (see Fig. 1) and there may be finally a loss. The gain is, in itself, very remarkable when it is remembered that the internal concentration of chloride is about $N/36$, while the external concentration in these solutions is only about $N/15{,}000$. Thus the chloride apparently enters against the normal diffusion gradient. The subsequent loss of chlorine may possibly be due to the cumulative effect of hydrogen-ions, *i.e.* a given number of hydrogen-ions absorbed by the protoplasm may affect it in the same way as does a high hydrogen-ion concentration. At any rate, this secondary effect is well marked in most of the cases examined. In No. 1, Table I, and Nos. 1 and 2, Table II, a similar effect occurs, so that the amount of chlorine lost increases very greatly between 32 and 50 hours.

THE DEATH POINT

The cases presented by the first examples in each series are particularly interesting because in each case the tissue was dead at the

end of 50 hours, and flabby and dying at the end of 32 hours. Moreover, these two cases, the only ones in which the tissue was killed, were the only ones in which the *p*H value of the cell sap was lowered in this series. It is noticeable that in both cases the mean of the external and internal *p*H values at death appears to be about *p*H 4·4. Death is shown in our estimations (*a*) by subsequent marked loss of chlorine, (*b*) by the flabbiness of the tissue, and (*c*) by its change in colour from cream to white. A cylinder of tissue at the "death point" usually shows a small cream core in the centre. Recognising the death point by these means then our results show the following relations between internal and external *p*H at the death point or in quite dead tissue:

Internal *p*H	External *p*H	Mean	External solution
4·8	4·0	4·4	Hydrochloric acid
4·7	4·2	4·45	
4·9	3·6	4·25	
4·4	3·8	4·1	
5·3	3·6	4·45	Hydrochloric acid + sodium chloride
5·0	3·7	4·35	
5·0	3·5	4·25	
4·2	4·0	4·1	Acetic acid + sodium chloride
4·8	4·0	4·4	
4·0	3·9	3·95	

In six additional cases, both internal and external *p*H values were below *p*H 4·0, the tissue having been exposed for a long time to the acid solution.

The colorimetric determinations of internal *p*H are probably not of a very high order of accuracy, owing to the turbidity of the juice, and the difficulty of separating moisture in the cell walls from the real cell sap. Nevertheless, these results are of great interest. They suggest that death occurs in acid solutions when the mean *p*H of the protoplasm is at or below *p*H 4·4, the external solution being of higher and the internal sap of lower hydrogen concentration. The *p*H value at which death ensues is, therefore, almost identical with the *p*H value below which rapid loss of chlorine takes place. Considering the possible shortcomings of the method of obtaining internal *p*H, this is so close an agreement as to suggest that the two facts are related.

Loss of Chlorine from Potato in the Presence of Sodium Chloride

It was pointed out in the preceding section that in very dilute solutions of hydrochloric acid, the chloride concentration becomes negligible and hence there ensues from the tissue a loss of chlorine

which is not due to hydrogen-ion concentration. To avoid this difficulty we made use of two sets of solutions:

(*a*) Those with constant amounts of sodium chloride and varying amounts of hydrochloric acid.

(*b*) Those with inversely proportional amounts of sodium chloride and hydrochloric acid, so that the total chlorine content of the solutions remained constant.

The results obtained with these two types of solution appear to show no difference at all and they will therefore be considered together. Tables IV and V deal with solutions of type (*a*), Table III deals with solutions of type (*b*), the variety of potato being the same as that used in Table IV.

TABLE III. Loss of Chlorine from 10 gms. potato in 100 c.c. solution

			5 hours			18 hours			43 hours		
No.	cc. of $N/10$ HCl	Initial pH	Solution pH	Chlorine lost (mgms.)	Juice pH	Solution pH	Chlorine lost (mgms.)	Juice pH	Solution pH	Chlorine lost (mgms.)	Juice pH
1	3·0	2·6	3·0	1·56	5·3	3·5	5·60	5·0	3·7	7·80	5·0
2	2·4	2·8	3·2	1·12	5·4	3·6	3·50	5·2	3·9	5·72	5·1
3	1·8	3·0	3·4	0·80	5·5	3·9	3·00	5·2	4·1	5·00	5·2
4	1·2	3·2	3·7	0·80	5·5	4·3	1·77	5·4	4·6	2·60	5·3
5	0·6	3·6	4·4	0·75	5·5	5·0	1·17	5·5	5·4	1·04	5·4
6	0·06	4·8	5·2	0·00	5·5	5·8	0·78	5·5	6·4	0·91	5·5
7	0·0	5·2	5·7	0·30	5·5	6·2	0·78	5·5	6·8	1·30	5·5

Total chlorine concentration of solution = 5 c.c. $N/10$ per 100 c.c., sodium chloride ($N/10$) added to make up required amount.

If the amount of sodium chloride is kept constant and the proportions of acid varied, then the highest external concentrations of chlorine occur in the more acid solutions. Whether the chlorine content varies or not the potato still continues to lose chlorine rapidly to the external solution if the pH of that solution is below about pH 4·4 at 4 or 5 hours. The "average pH" below which the loss takes place appears to be about pH 4·3 as shown in Fig. 1. One of the disadvantages of this method is that in practically all the solutions a loss of chlorine is shown, when sodium chloride is present. The results have therefore to be considered in terms of the "normal loss" of chlorine in solutions of pure sodium chloride of the given strength. When this is done, potato in all solutions below an average pH value of 4·3 shows a greater loss of chlorine than the normal and above this point it shows a normal or sub-normal loss. The results of Table IV are given in the form used in the graphs.

TABLE IV. Loss of Chlorine from 10 gms. potato in 100 c.c. of salt solution (*N*/200)

No.	Amount of *N*/100 HCl added c.c.	Initial *p*H	Chlorine lost in 5 hrs. (C) mgms.	*p*H of solution	Relative gain or loss of chlorine mgms.	"Average *p*H"
1	7·0	3·5	1·17	4·0	−0·78	3·75
2	5·0	3·6	1·56	4·2	−1·17	3·9
3	2·0	3·8	1·04	4·4	−0·65	4·1
4	0·7	4·4	0·26	4·6	0·13	4·5
5	0·0	5·0	0·39 (N)	5·0	0·0	5·0
6	0·3*	5·8	0·26	5·5	0·13	5·65
7	0·7*	7·4	0·26	6·8	0·13	7·1

* c.c. of *N*/100 NaOH.

The majority of the potatoes we have used give results like those in Tables III and IV. The set of results in Table V, however, were obtained with an unknown variety of potato which gave very small initial losses of chlorine. Great care was required in obtaining the figures given for 4 hours, as the differences were slight. The results obtained, however, are confirmed by those for 18 hours. Although the differences in this case are small, they confirm the results obtained in the earlier experiments.

So far as the potato is concerned these estimations appear to justify the conclusion that loss of chlorine is normal or sub-normal when the *p*H value of the external solution is 4·4 or above, while below this point the high hydrogen-ion concentration causes more rapid loss of chlorine. Further, the upper *p*H limit at which the death of the protoplasm occurs, seems to be very close to the same point so far as the available indications show.

TABLE V. Loss of Chlorine from potato (10 gms.) in 100 c.c. of sodium chloride solution

	Solution		4 hours		18 hours	
No.	c.c. of *N*/100 HCl added	*p*H	Chlorine lost by tissue mgms.	*p*H	Chlorine lost by tissue mgms.	*p*H
1	18·0	3·0	0·65	3·4	1·82	3·6
2	6·0	3·6	0·39	4·0	1·04	4·5
3	4·2	3·8	0·31	4·3	0·91	5·0
4	3·0	4·0	0·39	4·5	0·78	5·4
5	1·8	4·3	0·05	4·8	0·12	5·7
6	0·6	4·8	0·12	5·2	0·25	5·9
7	0·0	5·0	0·25	5·6	0·39	6·2
8	0·5*	6·5	0·12	6·4	0·25	6·4
9	1·0*	7·0	0·12	6·7	0·12	6·7
10	1·5*	8·2	0·0	7·0	0·12	7·0

* c.c. of *N*/100 NaOH.

The sap was *p*H 5·5 in this series. After 18 hours it fell in Nos. 1 and 2 to *p*H 5·3 and 5·4 respectively.

It is legitimate to consider the possible theoretical bearing of these facts. The plasma membrane appears to owe its properties partly to the presence of fats (see Clowes (2)) and partly to its protein content. No evidence is available which suggests that hydrogen-ion concentration would affect the fatty components of the protoplasmic membrane so as to increase the rate of diffusion of ions through this membrane below *p*H 4·4. The properties of the proteins are, on the other hand, intimately connected with the hydrogen-ion concentration[1] (see Loeb (6)). At some definite *p*H value a protein is iso-electric. On the acid side of this point it is positively charged and behaves as a base, on the alkaline side it is negatively charged and behaves as an anion. Further, at its iso-electric point, it is most easily precipitated and absorbs the minimum of water, hence possessing minimum volume at this point. The principal protein in potato is the globulin, *tuberin*, which is present in the expressed sap to the extent of between 1 and 2 per cent. The total protein content of potato is 8 per cent. (dry weight), and since potato contains about 80 per cent. of water (see above) almost all the protein present must be *tuberin*. In agreement with this conclusion is the fact that we have only been able to obtain very faint indications of the presence of any other proteins, in potato tissues.

The iso-electric point of tuberin lies between *p*H 4·3 and 4·5, a result obtained by Cohn, Gross and Johnson (3). We have confirmed this result for our potatoes (Pearsall and Ewing (7)), obtaining a value of *p*H 4·3–4·4 by the precipitation method. Thus potato with a sap reaction of about *p*H 5·6 normally lies on the alkaline side of the iso-electric point of its only important protein. As the results of the present paper show, rapid diffusion of chlorine from the cells takes place when the hydrogen-ion concentration of the external medium is increased to or beyond the iso-electric point of the chief protein. We are not for the moment concerned with the mechanism by which this increase in permeability is brought about. It is doubtless connected with precipitation of suspended protein or with contraction of a protein *gel*, though numerous other possibilities have also to be considered[2]. Of more importance is the possibility that

[1] The *lipins* may also have to be considered in this connection ultimately, but the quantities present in plant tissues appear to be very small.

[2] The increase in the amount of chlorine diffusing from the tissue does not appear to be due to its liberation from organic material in the presence of acid, since all the chlorine is ionic. By boiling and filtering crude potato sap to remove protein and then estimating the chlorine in the clear juice, it can be shown that below *p*H 4·5, 15 per cent. or more of the chlorine originally present is removed by the precipitate, presumably in combination with protein, while practically none is removed above *p*H 4·5.

the outward diffusion occurs at a *p*H value which coincides with the iso-electric point of the potato globulin, and that these two observed facts may not be necessarily connected. It seemed advisable, therefore, to examine other plant tissues to see if a similar relation existed between the rate of outward diffusion and the iso-electric point of the principal protein.

Carrot

We therefore examined the relation between the diffusion of chlorine from carrot and the hydrogen-ion concentration of the external medium. As the carrot is not a uniform tissue the cylinders of tissue were cut from the outer cortical region. The solutions used contained uniform concentrations of sodium chloride and varying proportions of hydrochloric acid or sodium hydrate. The results obtained are illustrated by the series in Table VI and Fig. 1. More rapid diffusion of chlorine after 4 hours' exposure takes place below *p*H 4·25 and not at *p*H 4·5. From the graphical results it is clear that normal diffusion would be found down to approximately *p*H 4·25. This is in close agreement with the iso-electric point of the carrot globulin as determined by Cohn, Gross and Johnson (3). Their figures, which we have confirmed, indicate a probable value of *p*H 4·3 for this protein. The normal reaction of the tissue is about *p*H 6·0.

Table VI. Diffusion of Chlorine into 100 c.c. of *N*/200 NaCl solution

	Solution		4 hours		18 hours	
No.	*p*H	Acid added *N*/10 HCl	*p*H	Chlorine lost by tissue mgms.	*p*H	Chlorine lost by tissue mgms.
1	3·0	2·1	3·2	0·78	3·4	1·43
2	3·2	1·4	3·4	0·65	3·7	1·17
3	3·5	0·7	3·8	0·78	4·3	1·17
4	3·8	0·5	4·2	0·52	4·9	0·65
5	4·2	0·2	4·5	0·13	5·3	0·13
6	4·8	0·1	5·0	0·13	5·6	0·39
7	5·2	0·0	5·5	0·26	6·0	0·39
8	6·2	0·05*	6·4	0·13	6·3	0·26
9	6·7	0·10*	6·7	0·26	6·7	0·26
10	7·0	0·15*	7·0	0·26	7·0	0·39

* c.c. of *N*/10 NaOH.

Nitella

A further example is presented by the alga *Nitella*. This plant possesses long single-celled internodes from which the sap can be extracted very simply. The data for the consideration of this case

are provided by Hoagland and Davis(5) in an extremely interesting paper. *Nitella* cultivated for several days in culture of varying hydrogen-ion concentrations showed injury when the *p*H value of the solution was at or below 4·4, the cell sap of plants in the latter solution having a reaction of *p*H 4·7. It was found that chlorine always diffused out of the cells in these cases, and it is pointed out that this outward diffusion of chlorine affords a delicate test for injury. We had previously determined the iso-electric point of *Nitella* protoplasm by the precipitation method and found that it lay at *p*H 4·6 (Pearsall and Ewing(7)). The normal reaction of *Nitella* sap is about *p*H 5·2.

It is clear that here again we have the same intimate connection between permeability and the iso-electric point, already demonstrated for potato and carrot.

APPLICATION OF RESULTS AND OF METHODS

The three cases considered agree in showing that ions may diffuse rapidly from living plant tissues brought to a hydrogen-ion concentration higher than that of the iso-electric point of the principal protein the tissue contains. Apart from the bearing of these results on our knowledge of the conditions affecting cellular permeability, they appear to be important because they suggest a method by which the iso-electric point of the protoplasm or the principal protein, can be determined in living tissues. It is thus possible to see, firstly, if the reaction of a given protein *in vitro* shows any relation to its behaviour in the living tissue or, secondly, to determine the tissue iso-electric point, without the necessity of extracting and isolating its proteins. The latter case is important because it is very difficult to obtain appreciable quantities of protein from many active plant tissues, and there is therefore little possibility of examining their protein reactions at first hand. Such a case is that of beet, from which we have been unable to obtain protein by any of the usual extraction methods. The fact that this tissue contains a cell-sap, coloured red by an anthocyanin, offers the possibility that the iso-electric point can be determined by finding the *p*H value at which rapid outward diffusion of this colour takes place.

The method employed consists of taking a series of cylinders of beetroot of uniform size and colour, and placing each in a solution of dilute acid or alkali. The particular acid used appears to have little or no effect on the result. We have used hydrochloric, acetic, nitric and citric acids. The tissue used should be fresh and turgid, and

preferably washed and allowed to stand in water a few hours previously. Beet which has been exposed to the air for some time becomes flaccid if uncut, and readily allows diffusion of the colour into almost any medium. It is then less easy to compare the results. Our solutions were made up with tap water. In Leeds tap water beet loses colour slightly, and this colour was taken as the standard of comparison. The colour of the external solution was always less than that in water at the following *p*H values:

After 24 hours

Colour negligible:

*p*H values: 10·5, 10·0, 9·6, 9·0, 7·5, 6·8, 6·5, 5·7, 5·6, 5·4, 5·3, 5·1, 5·3, 5·1, 4·9, 4·8

Colour decided but not deep:

*p*H values: 4·6, 4·6, 4·5, 4·5

All solutions more acid than these were deeply coloured, and the beet was also more or less flaccid. Solutions which were *p*H 4·5 or 4·6 at the end of 24 hours, were usually about *p*H 4·1–4·3 at the beginning of the experiment, and these values obviously lie just below the point at which rapid outward diffusion begins. The following additional series is instructive:

TABLE VII. Loss of colour by Beet in solutions of various *p*H values

No.	Initial *p*H	*p*H at 24 hours	Condition of solution
0	6·5	6·6	No colour
1	4·6	5·1	
2	4·3*	4·6	Coloured
4	3·9*	4·37	Deeply coloured
6	3·7	4·0	
8	3·6	—	
10	3·5	3·7	

* Losing colour most quickly in first half-hour.

The *p*H values of such coloured solutions were, of course, determined electrometrically. In this case, initial loss starts at *p*H 4·3, not at *p*H 4·6, but the tissue loses less colour between *p*H 4·3 and 4·6 than when below *p*H 4·3 all the time. The point required, therefore, clearly lies between *p*H 4·3 and 4·6. From another series it is found that beet starting at *p*H 4·4 and finishing 24 hours later at *p*H 4·8 loses practically no colour. Thus if no colour is lost at *p*H 4·4 and some is lost at *p*H 4·3, between *p*H 4·3 and 4·4 is approximately the critical point at which rapid outward diffusion begins. This is therefore assumed to be the iso-electric point of the beet protein.

It should, perhaps, be added that we have obtained indications by other methods (undescribed) which give an iso-electric point for beet tissue in the same region, about *p*H 4·3–4·4.

SUMMARY

(1) Evidence is presented to show that rapid outward diffusion of chlorine-ions ensues when living plant tissue is brought to a hydrogen-ion concentration equal to or greater than that at which its chief protein (potato, carrot) or protoplasm (*Nitella*) is iso-electric.

(2) It is suggested that the methods employed can be used to determine the iso-electric point of the protoplasm in tissues from which extraction of protein is difficult.

(3) The outward diffusion of coloured sap from beet becomes rapid at or below *p*H 4·3. This is assumed to be the approximate position of the iso-electric point for the protoplasm of this tissue.

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FLOWERING IN THE NORTH OF ENGLAND IN 1922 AND 1923

By R. H. McCREA, B.Sc.

(With 4 figures in the text)

THE following is a brief phænological study for the years 1922 and 1923 of the district of N.E. Derbyshire for the most part, but, in the late summer, of the neighbourhood of Whitby, Yorkshire.

The original intention was to correlate the influence of various atmospheric factors with the time of flowering, the factors chosen being those emphasised by Warming for their ecological importance (*Oecology of Plants*, Eng. ed., Oxford, 1909), namely, temperature, sunshine, rain and wind. The analysis was attempted, but the results obtained were considered to be too indefinite and the data too bulky to justify publication, so that the course adopted by Sir F. Darwin has been followed; that is, to publish the phænological lists along with the records of temperature, "which is the principal condition affecting the rate of flowering" ("Studies in Phænology," No. 2, 1920, *New Phytologist*, **20**, p. 30).

This is strikingly illustrated by a comparison of the flowering and temperatures for the two years under consideration. Some very striking differences are noticeable; the flowering for the early part of 1923 being much ahead of that for the same period of 1922, which corresponds very well with the higher mean temperature of the air for that period in 1923; and again the falling off later of the flowering for 1923 below that of 1922 and its subsequent recovery correspond to a similar drop and subsequent rise in the air temperature over approximately the same periods.

The comparison of the flowering was effected first by selecting those plants which occurred in the lists for both years, and then comparing the number which had already flowered at corresponding times in the two years. The mean temperatures per week are those supplied by the official observer at Worksop, Nottinghamshire, which was selected as the most representative observation centre available.

The results are shown graphically (Fig. 1). The graph of mean temperatures (Fig. 2) over the same periods is added to bring out the general correspondence in the two cases. The actual mean temperatures rather than the temperature summations are given, as the

TABLE I

1922				1923			
Week No.	Week commencing	Mean temp. ° F.	No. of species already flowered	Week No.	Week commencing	Mean temp. ° F.	No. of species already flowered
1	Jan. 1	40·5	1	1	Dec. 31'22	40·3	2
2	8	40·0	1	2	Jan. 7	41·1	3
3	15	30·7	1	3	14	42·2	3
4	22	35·3	1	4	21	50·1	8
5	29	40·6	1	5	28	41·9	12
6	Feb. 5	31·2	1	6	Feb. 4	41·4	18
7	12	37·5	2	7	11	40·0	18
8	19	46·1	6	8	18	33·1	19
9	26	45·3	8	9	25	44·1	19
10	March 5	43·2	10	10	March 4	42·0	21
11	12	40·6	13	11	11	41·1	26
12	19	37·1	14	12	18	43·5	28
13	26	36·9	18	13	25	47·8	36
14	April 2	37·4	19	14	April 1	46·2	40
15	9	44·1	23	15	8	45·7	47
16	16	42·5	26	16	15	42·8	56
17	23	44·1	33	17	22	44·5	59
18	30	47·0	34	18	29	54·9	63
19	May 7	50·9	50	19	May 6	48·4	68
20	14	54·9	65	20	13	45·9	72
21	21	62·5	80	21	20	48·9	75
22	28	61·2	90	22	27	47·7	82
23	June 4	57·3	103	23	June 3	53·1	88
24	11	54·8	114	24	10	54·3	100
25	18	56·8	122	25	17	56·4	105
26	25	54·7	128	26	24	56·6	117
27	July 2	55·3	137	27	July 1	63·3	124
28	9	55·8	139	28	8	68·8	131
29	16	58·0	140	29	15	63·8	136
30	23	56·6	143	30	22	61·5	145
31	30	57·8	147	31	29	59·4	148
32	Aug. 6	53·6	149	32	Aug. 5	62·5	150
33	13	57·3	150	33	12	60·6	150
34	20	56·6	150	34	19	58·1	150
35	27	57·1	150	35	26	54·5	150

latter would involve many complications in such a heterogeneous collection of species. The means for four-weekly periods are plotted so as to avoid the minor fluctuations of shorter periods.

Deviations from the Normal

The foregoing method of treatment is, of course, purely empirical: a more logical procedure would be to take the deviations from the normal of the mean temperatures and of the flowering over the periods considered. The temperature, etc. deviations are available in the "Weekly Weather Reports" of the Meteorological Office, and

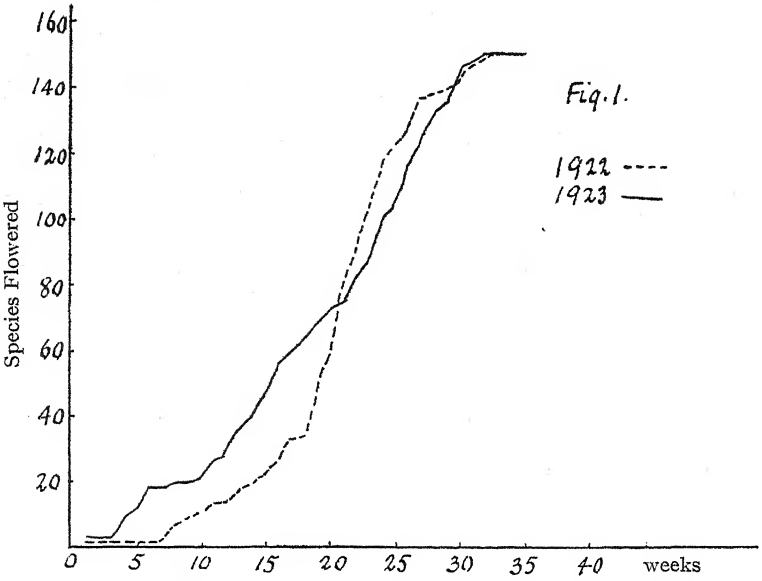

Fig. 1. Showing the number of species, out of a selected number, which had flowered at the same dates in 1922 and 1923.

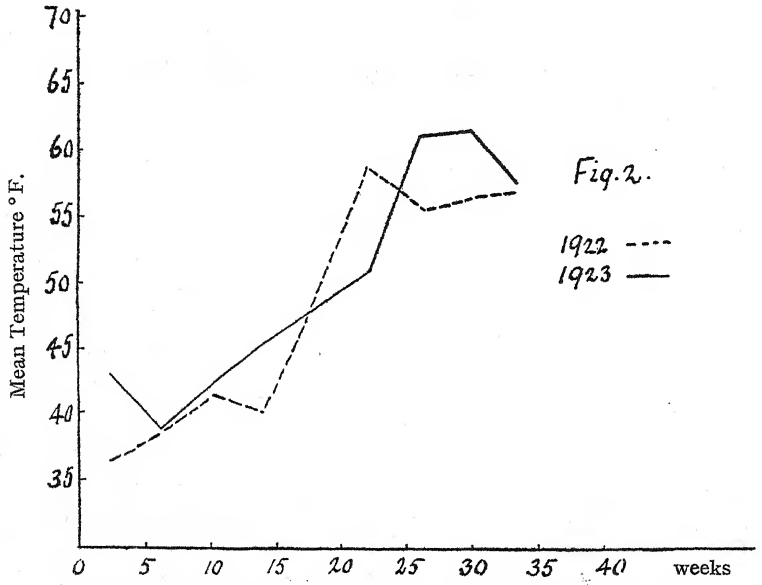

Fig. 2. The mean 4-weekly temperatures over the same periods in 1922 and 1923.

the normal flowering would strictly demand the mean of observations over a long period of years; but, in default of this, the mean was taken of the two years under consideration. This would ordinarily, no doubt, be a most rash proceeding, but it will be noted that both the temperature and the flowering records of these two years were, for the most part, strikingly opposed to each other. Hence it was thought that the expedient of determining the weekly deviations in the flowering of species from this mean would be justified as a first approximation. The results are recorded in the graphs (Figs. 3 and 4), and the agreement between temperature and flowering deviations is, at least in general, apparent.

As far as possible the mean temperatures were taken from the records of Blackpool and Scarborough respectively, when the plants were found in the Morecambe district or in that south of Whitby.

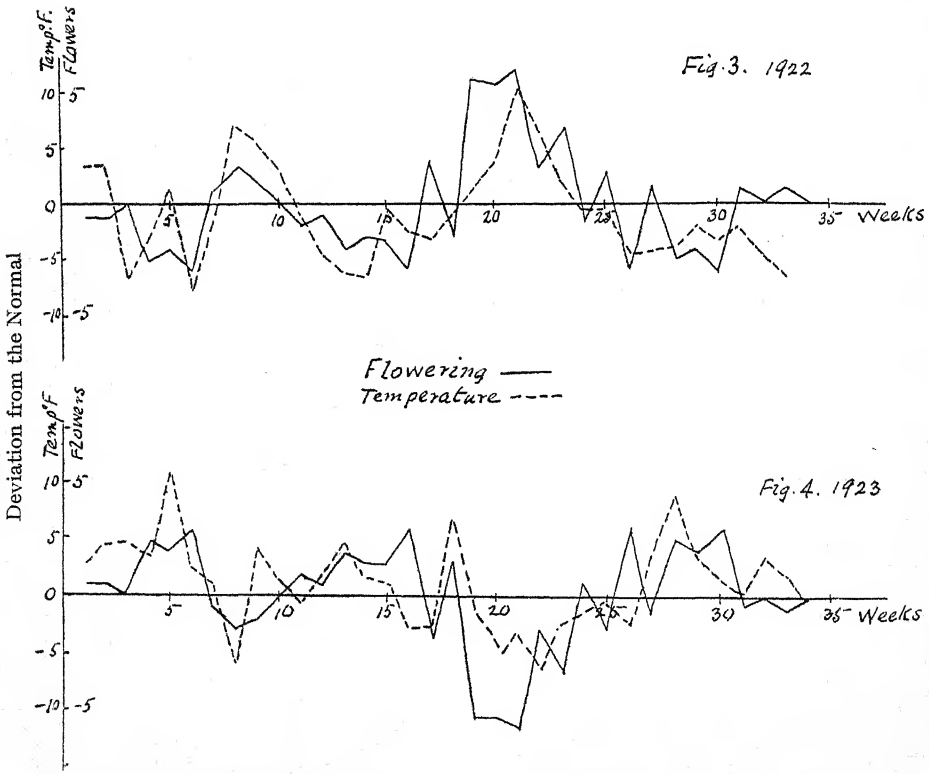

Fig. 3. Showing the deviations from the normal of the temperature and the flowering for 1922. Fig. 4. The same for 1923.

TABLE II

1922			1923		
Week No.	Flowering deviation from normal	Temperature deviation from normal ° F.	Week No.	Flowering deviation from normal	Temperature deviation from normal ° F.
1	−0·5	+ 3·1	1	+0·5	+ 2·9
2	−0·5	+ 3·2	2	+0·5	+ 4·3
3	0	− 6·9	3	0	+ 4·6
4	−2·5	− 3·1	4	+2·5	+ 3·5
5	−2·0	+ 1·6	5	+2·0	+11·1
6	−3·0	− 7·6	6	+3·0	+ 2·6
7	+0·5	− 1·5	7	−0·5	+ 1·0
8	+1·5	+ 7·3	8	−1·5	− 5·7
9	+1·0	+ 5·6	9	−1·0	+ 4·4
10	0	+ 2·9	10	0	+ 1·7
11	−1·0	− 1·1	11	+1·0	− 0·6
12	−0·5	− 4·5	12	+0·5	+ 1·9
13	−2·0	− 6·1	13	+2·0	+ 4·8
14	−1·5	− 6·8	14	+1·5	+ 2·0
15	−1·5	− 0·6	15	+1·5	+ 1·1
16	−3·0	− 2·5	16	+3·0	− 3·0
17	+2·0	− 3·2	17	−2·0	− 2·8
18	−1·5	− 1·0	18	+1·5	+ 6·9
19	+5·5	+ 1·3	19	−5·5	− 1·2
20	+5·5	+ 3·8	20	−5·5	− 5·2
21	+6·0	+10·1	21	−6·0	− 3·5
22	+1·5	+ 6·6	22	−1·5	− 6·9
23	+3·5	+ 1·6	23	−3·5	− 2·6
24	−0·5	− 0·8	24	+0·5	− 1·3
25	+1·5	− 0·4	25	−1·5	− 0·8
26	−3·0	− 4·3	26	+3·0	− 2·4
27	+1·0	− 4·2	27	−1·0	+ 3·8
28	−2·5	− 4·0	28	+2·5	+ 9·0
29	−2·0	− 2·2	29	+2·0	+ 3·6
30	−3·0	− 3·2	30	+3·0	+ 1·7
31	+0·5	− 2·2	31	−0·5	+ 0·2
32	0	− 4·9	32	0	+ 3·5
33	+0·5	− 6·6	33	−0·5	+ 2·0
34	0	− 0·2	34	0	− 0·9
35	0	—	35	0	− 2·9

It is not, of course, assumed here that other climatic influences besides temperature do not play an important part in the earliest flowering of the plant, but it does not appear that plants react as quickly to them. The physiological reasons are fairly clear. To mention only one; we know that the speed of a chemical reaction is commonly doubled for a rise in temperature of about 10° C. (F. F. Blackman, "Photosynthesis," *Nature*, No. 2773, Dec. 23, 1922). Hence enzyme action, and the general metabolism of the plant, must be greatly speeded up by a rise in mean temperature.

PHÆNOLOGICAL TABLES, 1922

In the following lists the letter *M* or *W* prefixed to a plant signifies that it was found in the neighbourhood of Morecambe or of Whitby respectively.

TABLE III

No.	1922	Name
1	Jan. 3	Corylus avellana (♂)
2	Feb. 16	Galanthus nivalis
3	23	Primula vulgaris
4	24	Tussilago farfara
5	25	Ranunculus ficaria
6	„	Ulmus montana
7	26	Corylus avellana (♀)
8	28	Alnus rotundifolia
9	Mar. 6	Scilla verna
10	„	Salix caprea
11	12	Mercurialis perennis
12	„	Salix nigra
13	18	Veronica agrestis
14	25	Potentilla fragariastrum
15	26	Prunus spinosa
16	29	Anemone nemorosa
17	„	Viola odorata
18	Apr. 1	Vinca minor
19	„	Caltha palustris
20	4	Viola canina
21	13	Arum maculatum
22	14	Narcissus pseudonarcissus
M 23	15	Cytisus scoparius
M 24	„	Cardamine hirsuta
M 25	17	Lamium purpureum
26	„	Scilla nutans
M 27	21	Oxalis acetosella
28	23	Veronica hederæfolia
29	„	Luzula campestris
30	24	Chrysoplenium oppositifolium
31	„	Primula veris
32	25	Adoxa moschatellina
33	29	Nepeta hederacea
34	„	Galium cruciata
35	„	Luzula sylvatica
36	May 6	Stellaria holostea
37	7	Cardamine pratensis
38	„	Ranunculus bulbosus
39	„	Lathyrus macrorrhizus
40	„	Prunus cerasus
41	„	Fraxinus excelsior
42	10	Viola tricolor
43	„	Lamium album
44	„	Anthriscus cerefolium
45	12	Cratægus oxyacantha
46	13	Lamium galeobdolon
47	„	Alliaria officinalis
48	„	Plantago lanceolata
49	„	Montia fontana
50	„	Poa annua
51	„	Fragaria vesca
52	„	Ranunculus aquatilis
53	May 13	Veronica chamædrys
54	14	Allium ursinum
55	„	Corydalis claviculata
56	„	Corydalis lutea
57	„	Quercus sessiliflora
58	16	Pyrus malus
59	17	Cerastium vulgatum
60	19	Alchemilla vulgaris
61	„	Viola palustris
62	20	Saxifraga tridactylites
63	„	Carex glauca
64	„	Draba verna
65	„	Veronica arvensis
66	„	Acer pseudo-platanus
67	„	Ajuga reptans
68	„	Asperula odorata
69	„	Vicia sepium
70	„	Luzula pilosa
71	„	Euphorbia helioscopia
72	„	Linaria cymbalaria
73	„	Orchis mascula
74	„	Geranium lucidum
75	„	Ajuga genevensis
76	„	Vaccinium myrtillus
77	21	Anchusa sempervirens
78	„	Rumex acetosa
79	„	Arenaria verna
80	22	Lychnis diurna
81	„	Conopodium denudatum
82	„	Trifolium pratense
83	„	Alopecurus pratensis
84	„	Acer campestre
85	„	Brassica sinapis
86	„	Heracleum sphondylium
87	„	Lychnis vespertina
88	„	Myosotis arvensis
89	23	Ilex aquifolium
90	„	Geranium molle
91	„	Medicago lupulina
92	24	Veronica serpyllifolia
93	„	Arenaria serpyllifolia
94	„	Lotus corniculatus
95	„	Rhinanthus carist-galli
96	25	Potentilla tormentilla
97	„	Lysimachia nemorum
98	26	Pyrus aucuparia
99	„	Geranium robertianum
100	27	Arabis hirsuta
101	„	Polygala vulgaris
102	„	Veronica beccabunga
103	„	Sagina procumbens
104	„	Galium aparine

TABLE III (*continued*)

No.	1922	Name
105	May 28	Berberis vulgaris
106	29	Chrysanthemum leucanthemum
107	,,	Leontodon hispidus
108	31	Cardamine amara
109	,,	Stellaria graminea
110	,,	Sisymbrium officinale
111	,,	Lolium perenne
112	,,	Dactylis glomerata
113	June 1	Hieracium pilosella
114	,,	Potentilla reptans
115	,,	Rubus idæus
116	3	Rubus fruticosus
117	,,	Vicia cracca
118	4	Aquilegia vulgaris
119	,,	Potentilla palustris
120	,,	Sanicula europea
121	,,	Viburnum opulus
122	,,	Poterium sanguisorba
123	,,	Epilobium montanum
124	5	Rosa canina
125	6	Plantago media
126	,,	Polygonum bistorta
127	,,	Nasturtium officinale
128	7	Melica uniflora
129	,,	Sedum acre
130	,,	Fumaria officinalis
131	,,	Poa pratensis
132	,,	Lepidium Smithii (?)
133	10	Papaver rhœas
134	,,	Geranium pratense
135	,,	Convolvulus sepium
136	,,	Prunella vulgaris
137	12	Scrophularia nodosa
138	,,	Lathyrus pratensis
139	,,	Chenopodium bonus-henricus
140	,,	Symphytum officinale
141	13	Stachys sylvatica
142	,,	Reseda lutea
143	,,	Anagallis arvensis
144	14	Digitalis purpurea
145	,,	Crepis virens
146	15	Urtica dioica
147	,,	Plantago major
148	,,	Holcus lanatus
149	,,	Euphorbia exigua
150	16	Cirsium palustre
151	18	Centaurea nigra
152	,,	Malva sylvestris
153	20	Sonchus oleraceus
154	21	Achillea millefolium
155	,,	Rosa arvensis
156	22	Veronica officinalis
157	,,	Lonicera periclymenum
158	23	Lapsana communis
159	24	Vicia sativa
160	June 24	Polygonum aviculare
161	27	Erica cinerea
162	28	Teucrium scorodonia
163	,,	Epilobium angustifolium
164	,,	Conium maculatum
165	July 1	Lychnis flos-cuculi
166	,,	Hypericum humifusum
167	,,	Ranunculus lingua
168	,,	Scabiosa arvensis
169	3	Sanguisorba officinalis
170	4	Galium verum
171	,,	Æthusa cynapium
172	,,	Erica tetralix
173	,,	Galium uliginosum
174	5	Stachys betonica
175	,,	Epilobium tetragonum
176	6	Convolvulus arvensis
177	,,	Ligustrum vulgare
178	,,	Galeopsis tetrahit
179	7	Carduus eriophorus
180	8	Spiræa ulmaria
181	,,	Orchis maculata
182	,,	Senecio aquaticus
183	,,	Senecio jacobæa
184	10	Senecio viscosus
185	12	Campanula rotundifolia
186	,,	Origanum vulgare
187	13	Nuphar luteum
188	,,	Alisma plantago
189	18	Circæa lutetiana
190	,,	Melampyrum pratense
191	23	Polygonum persicaria
192	25	Filago germanica
193	26	Chenopodium album
194	,,	Spergularia rubra
W 195	,,	Erythræa centaurium
196	27	Hypericum quadrangulum
197	29	Epilobium hirsutum
198	,,	Pimpinella saxifraga
199	30	Malva moschata
200	,,	Caucalis anthriscus
W 201	,,	Hypericum androsæmum
202	Aug. 1	Tanacetum vulgare
203	,,	Angelica sylvestris
204	,,	Solidago virga-aurea
205	2	Atriplex patula
206	3	Campanula trachelium
207	,,	Calluna vulgaris
208	6	Fœniculum vulgare
209	7	Scabiosa succisa
W 210	,,	Inula dysenterica
211	13	Gnaphalium sylvaticum
W 212	16	Drosera rotundifolia
213	—	—
214	—	—

Phænological Tables, 1923

In the following lists the letter *W* prefixed to a plant signifies that it was found near Whitby; otherwise it occurred in the N.E. Derbyshire district.

Table IV

No.	End of 1922		Name
1	Dec.	31	Bellis perennis
2		,,	Chrysanthemum leucanthemum
3		,,	Ulex europæus
4		,,	Senecio vulgaris
5		,,	Taraxacum officinale
6		,,	Ranunculus acris
7		,,	Anthemis arvensis
8		,,	Chærophyllum sylvestre
9		,,	Lamium album
	1923		
10	Jan.	1	Capsella bursa-pastoris
11		7	Stellaria media
12		14	Veronica agrestis
13		,,	Omphalodes verna
14		21	Vinca minor
15		23	Sonchus oleraceus
16		24	Corylus avellana (♂ and ♀)
17		27	Mercurialis perennis
18		28	Galanthus nivalis [wild]
19		29	Crocus vernus [garden]
20		31	Ranunculus ficaria
21	Feb.	1	Potentilla fragariastrum
22		3	Viola canina
23		4	Tussilago farfara
24		,,	Lychnis diurna[1]
25		,,	Primula vulgaris
26		,,	Viola odorata
27		9	Ulmus montana
28		,,	Alnus rotundifolia
29		20	Scilla verna
30	Mar.	4	Salix caprea (♂)
31		,,	Poa annua
32		14	Caltha palustris
33		16	Adoxa moschatellina
34		17	Lamium purpureum
35		,,	Cardamine hirsuta
36		,,	Anemone nemorosa
37		20	Tussilago petasites
38		23	Draba verna
39		24	Nepeta hederacea
40		25	Saxifraga tridactylites
41		,,	Fragaria vesca
42		,,	Prunus cerasus
43		26	Luzula campestris
44		27	Myosotis sylvatica
45		29	Lamium galeobdolon
46		,,	Arenaria serpyllifolia
47		31	Veronica hederæfolia
48		,,	Myosotis arvensis
49	Apr.	4	Chrysoplenium oppositifolium
50		5	Ranunculus auricomus

No.	1923		Name
51	Apr.	7	Stellaria holostea
52		,,	Viola tricolor
53		,,	Cerastium vulgatum
54		9	Scilla nutans
55		10	Ranunculus bulbosus
56		,,	Cardamine pratensis
57		,,	Oxalis acetosella
58		11	Corydalis claviculata
59		13	Primula veris
60		14	Montia fontana
61		15	Vaccinium vitis-idæa
62		,,	Galium cruciata
63		,,	Anchusa sempervirens
64		,,	Lathyrus macrorrhizus
65		16	Alliaria officinalis
66		,,	Ranunculus lenormandi
67		,,	Viola palustris
68		18	Asperula odorata
69		,,	Vaccinium myrtillus
70		21	Geum urbanum
71		23	Veronica chamædrys
72		,,	Veronica arvensis
73		24	Plantago lanceolata
74		29	Arum maculatum
75	May	1	Allium ursinum
76		2	Carex panicea
77		3	Ranunculus repens
78		,,	Vicia sepium
79		,,	Trifolium minus
80		4	Fraxinus excelsior
81		5	Orchis morio
82		6	Cratægus oxyacantha
83		,,	Cytisus scoparius
84		,,	Alopecurus pratensis
85		8	Ajuga reptans
86		,,	Veronica serpyllifolia
87		9	Potentilla reptans
88		10	Veronica montana
89		,,	Myosotis versicolor
90		,,	Arabis perfoliata
91		12	Alchemilla vulgaris
92		13	Potentilla anserina
93		14	Ajuga genevensis
94		16	Trifolium pratense
95		,,	Polygala vulgaris
96		,,	Pedicularis sylvatica
97		17	Conopodium denudatum
98		,,	Geranium molle
99		18	Geranium robertianum
100		19	Rumex acetosella
101		,,	Rumex obtusifolius
102		,,	Rumex acetosa

[1] Last year's plant.

TABLE IV (*continued*)

No.	1923	Name
103	May 19	Melica uniflora
104	20	Symphytum officinale
105	,,	Potentilla tormentilla
106	21	Dactylis glomerata
107	24	Rhinanthus crista-galli
108	27	Lepidium Smithii (?)
109	29	Stellaria uliginosa
110	31	Veronica beccabunga
111	June 1	Chenopodium bonus-henricus
112	,,	Sanicula europea
113	,,	Lotus corniculatus
114	,,	Aquilegia vulgaris
115	2	Berberis vulgaris
116	3	Trifolium repens
117	,,	Sisymbrium officinale
118	6	Reseda lutea
119	,,	Polygonum bistorta
120	8	Lysimachia nemorum
121	,,	Hypochœris radicata
122	,,	Veronica officinalis
123	9	Chrysanthemum leucanthemum
124	,,	Aethusa cynapium
125	,,	Scandix pecten
126	,,	Chærophyllum temulum
127	12	Linaria cymbalaria
128	,,	Hieracium pilosella
129	13	Stellaria graminea
130	14	Corydalis lutea
131	,,	Rubus cæsius
132	,,	Viburnum opulus
133	,,	Lolium perenne
134	15	Leontodon hispidus
135	,,	Scrophularia nodosa
136	,,	Epilobium montanum
137	,,	Silene cucubalus
138	,,	Tamus communis
139	,,	Galium saxatile
140	,,	Ranunculus arvensis
141	16	Sambucus nigra
142	,,	Urtica dioica
143	,,	Crepis virens
144	,,	Galium aparine
145	,,	Rubus idæus
146	18	Chelidonium majus
147	19	Crepis taraxacifolia
148	20	Rosa canina
149	,,	Solanum dulcamara
150	,,	Anagalus arvensis
151	21	Euphorbia peplus
152	23	Rubus fructicosus
153	,,	Lychnis flos-cuculi
154	,,	Prunella vulgaris
155	,,	Carex pendula
156	25	Heracleum sphondyllium
157	,,	Melampyrum pratense
158	,,	Spergularia rubra
159	26	Ranunculus lingua
160	,,	Scrophularia aquatica
161	,,	Papaver rhœas
162	27	Stachys sylvatica
163	June 27	Hieracium murorum
164	,,	Plantago media
165	,,	Erica cinerea
166	,,	Sedum acre
167	28	Digitalis purpurea
168	,,	Cirsium palustre
169	29	Polygonum aviculare
170	,,	Lonicera periclymenum
171	30	Lathyrus pratensis
172	,,	Briza media
173	,,	Aconitum napellus
174	July 1	Spergularia arvensis
175	4	Delphinium ajacis
176	,,	Ulmaria palustris
177	5	Rosa arvensis
178	,,	Hypericum humifusum
179	6	Geranium pratense
180	,,	Scabiosa arvensis
181	7	Lapsana communis
182	,,	Lychnis githago
183	,,	Achillea millefolium
184	8	Vicia cracca
185	,,	Carduus nutans
186	,,	Linaria vulgaris
187	9	Plantago major
188	,,	Epilobium angustifolium
189	,,	Malva sylvestris
190	10	Thymus serpyllum
191	11	Salvia pratensis
192	,,	Verbascum thrapsus
193	,,	Hypericum pulchrum
194	12	Stachys betonica
195	,,	Circæa lutetiana
196	,,	Cirsium arvense
197	,,	Trifolium medium
198	13	Valeriana officinalis
199	,,	Sanguisorba officinalis
200	14	Origanum vulgare
201	15	Campanula rotundifolia
W 202	,,	Daucus carota
W 203	,,	Agrimonia eupatoria
W 204	,,	Senecio sylvaticus
W 205	,,	Vicia sylvatica
206	16	Sedum album
207	18	Fœniculum vulgare
208	19	Galium verum
209	20	Centaurea nigra
W 210	,,	Habenaria conopsea
211	21	Ligustrum vulgare
212	22	Senecio Jacobæa
W 213	23	Hypericum hirsutum
W 214	,,	Poterium sanguisorba
W 215	25	Galeopsis tetrahit
W 216	26	Hypericum androsæmum
W 217	,,	Linum catharticum
W 218	,,	Calystegia sepium
W 219	,,	Polygonum amphibium
W 220	,,	Senecio aquaticus
W 221	,,	Epilobium hirsutum
W 222	27	Convolvulus arvensis

TABLE IV (*continued*)

No.	1923	Name
W 223	July 27	Nymphæa lutea
W 224	28	Centaurium capitatum
W 225	,,	Euphrasia officinalis
W 226	,,	Solidago virga-aurea
W 227	,,	Arctium vulgare
W 228	,,	Ononis repens
W 229	,,	Orchis pyramidalis
W 230	29	Parnassia palustris
W 231	,,	Eupatorium cannabinum
W 232	,,	Hypericum quadrangulum
W 233	31	Tanacetum vulgare
W 234	July 31	Carlina vulgaris
W 235	Aug. 1	Medicago sativa
W 236	2	Scabiosa succisa
W 237	6	Calluna vulgaris
W 238	7	Pulicaria dysenterica
W 239	9	Achillea ptarmica
W 240	10	Tilia europea
W 241	12	Drosera rotundifolia
W 242	13	Bartsia odontites
W 243	14	Polygonum lapathifolium

In conclusion I wish to thank very sincerely Mr Howarth, Curator of the Weston Park Museum, and Mr A. P. Hunt, Librarian to the University, Sheffield, for their kindness in lending the Weekly Weather Reports for 1922 and 1923; also Colonel H. Mellish of Hodsock Priory, Worksop, for his marked courtesy in sending me direct the mean temperature data for Worksop; and last, but not least, my son Andrew, for his indefatigable labours in the field, and his help in the preparation of this paper for publication.

A FUERTEVENTURA DIARY

BY R. LLOYD PRAEGER, D.Sc.

FUERTEVENTURA is, I think, the least known of the seven islands which make up the Canary Archipelago. It is also the least occupied by vegetation, whether in the form of native plants or of crops—not on account of sterility, for over wide areas the soil is amazingly fertile, but by reason of the inadequacy of the rainfall. The two eastern islands of the Canary group, Fuerteventura and Lanzarote, indeed, recall the Sahara, which lies about a hundred miles to the eastward, as much as they do the islands of Grand Canary and Teneriffe, which lie about the same distance to the west. Although Lanzarote is nearer to the African coast than Fuerteventura, it is rather more verdant, on account of its greater average elevation and consequent increased precipitation. On Fuerteventura the people think themselves lucky if they get good rain twice in the year, and this amount suffices to yield excellent crops. The average annual rainfall is 5 or 6 inches: this falls mainly during the winter months.

Some notes on the vegetation of the island as seen during a brief visit last spring may be of interest. Desert conditions were em-

phasised at the time of my visit, for the winter rains had failed. Famine was in prospect, and the people and their stock were leaving the island. Water, food and lodging were very difficult to procure; and the large section of the flora which consists of annual plants was conspicuous only by its absence. I have left my notes in the diary form in which they were written.

March 14. Gran Tarajal at dawn. A sandy beach backed with ragged Tamarisk and a cluster of eastern-looking, white, flat-roofed houses. Behind, a flat valley with a few palms and windmill pumps. On either hand, bare stony hills, descending steeply into the sea, and apparently quite devoid of vegetation. On shore at sunrise. A few Chenopodiaceæ mingle with the Tamarisks on the flat. On the lower slopes of the hills just two plants, rare and stunted—*Zollikoferia spinosa* Boiss. and *Nicotiana glauca* L.—the former scattered thinly, the latter in the water-courses: these species, and their distribution as indicated, proved very characteristic of the desert parts of the whole Canary group, as of the adjoining parts of the African mainland. After delays proper to the country and the people we got away—two camels, two drivers, a wolfy dog, my wife and myself, and Dr Burchard of Orotava, whose knowledge of the country, the people, and the plants proved of much service. All day we plodded southward through lonely hills over mere desert, desolate and done for; a very sparse vegetation of Zollikoferia, Nicotiana, *Lycium afrum* Reich. The annual and perennial herbs that should brighten the ground in mid-spring almost entirely absent on account of the drought; but the pretty bird-like blossoms of *Linaria heterophylla* Spreng. were here and there, with silvery small patches of *Lotus lancerottensis* W.B. var. *villosa* W.B. and close tufts of *Helianthemum canariense* Pers. with lemon-yellow flowers. One tiny oasis of bright green crops where in a hollow there is a deep accumulation of loam, and a windmill pump raises the water which is conducted through every portion of each plot. After dark we reach Matas Blancas, a collection of fishermen's huts along the edge of a low bluff; mostly half dug-out. Accommodation not attractive, so we retire to the sandy beach with sleeping-bags.

March 15. The mountainous peninsula of Handia is joined to the main part of the island by a broad isthmus entirely sandy though by no means low, the sea-sand being blown up and over hills of nearly a thousand feet in height. Our course lies along the strand on the eastern side. The desert plants of yesterday represent almost the only vegetation, save on low clayey flats, sometimes sand-covered,

occupied by grey stretches of shrubby Chenopodiaceæ—*Salicornia fruticosa* L., *Sueda fruticosa* Forsk. and *vermiculata* Forsk., *Atriplex glauca* L.; also some *Citrullus Colocynthus* Schrad. and *Euphorbia Paralias* L. These grew sometimes on low dunes, formed by the accumulation of sand about their own stems and branches, sometimes in a flat heath-like sward. On the rising ground Zollikoferia still maintained its dominance, and little else was seen either on the sand-covered hills or on the stony mountain-slopes which at length succeeded them. The sandy beach continued to Morro del Jable, a village which proclaimed itself afar off by an overpowering odour of drying fish, so that we again preferred the shore as a bed—and on several following nights.

March 16. At length we were within striking distance of the high ground we had come to see—the famous Orejas de Asno (Asses' Ears), so called from their outline as seen from the north-east. Most of the maps are quite wrong about the geography of Handia. A high ridge runs along the northern coast, magnificently precipitous on the northern side and broken into sharp peaks along its axis. On the other side, long parallel ridges, with deep valleys between, run down to the southern shore. Up one of these, through a scrub of *Lycium afrum*, our camels now pick their way. As we ascend, the Lycium becomes greenish-grey, as a better supply of moisture encourages it to break into leaf. At 500 feet *Euphorbia ? obtusifolia* Poir. comes in, becoming very common and covering the hill-sides with bushes of pale green. It is joined by *Kleinia neriifolia* Haw., growing up to 6 feet high, with thick grey branches. At this elevation both it and the Euphorbia are in full leaf. At 800 feet the beautiful *Asteriscus sericeus* DC. begins, becoming very abundant upwards, and covering the ground with silvery mounds as yet only sparsely decked with the great golden flower-heads. Meanwhile small annuals and perennials have been increasing in number, and at 800 feet the ground is full of flowers—sheets of the fragile *Asphodelus tenuifolius* Cav. (especially in the torrent bed), the handsome *Picridium tingitanum* Sch. Bip. like a golden dandelion with a purple eye, and species (in many cases endemic in Fuerteventura) of Ononis, Trifolium, Tolpis, Senecio, Illecebrum, etc. Zollikoferia, *Euphorbia ?obtusifolia* and *Lycium afrum*, all essentially lowland, gave out at about 1000 feet. Kleinia continued to 2000 feet. We had been promised a sight of running water—an extraordinary phenomenon on this island—and were taken to a rocky slope at 1500 feet, where, fenced about by groves of gigantic *Euphorbia canariensis* L., a tiny trickle emerged from a crack

and flowed for a few yards through a sward of dwarf *Apium graveolens* L., *Senebiera didyma* Pers., *Nasturtium officinale* R.Br. and *Spergularia rubra* Pers. Thence along a windy ridge through groves of *Asteriscus sericeus*, getting on the way a number of small herbs. The summit (Pico de la Zarza, 2770 feet) comes with abruptness, and we are on the edge of a great precipice draped with shrubs, herbs, ferns and mosses—a surprising vegetation for this desert island. To right and left the scarp runs for miles in a succession of tall peaks; below, across a couple of miles of parched farm-land seamed with dry water-courses, lies the Atlantic. It is extremely difficult to botanise here, as most of the ground is quite impossible; we can but nibble a little on small ledges at the top of the cliff. The vegetation is most interesting. I am indebted to Dr Burchard for the following list of the rarer plants which occur: in reading it the desert condition of almost the entire island has to be kept in mind:

Aichryson pachycaulon Bolle
Bupleurum canescens Schousb var. handiensis Bolle } Only known station
Asplenium Adiantum-nigrum L. var. acutum Bory
Ononis Christii Bolle
Alsine platyphylla J. Gay
Andryala pinnatifida Ait.
Barkhausia hieracioides Lowe
Carduus bœocephalus Webb var. Bourgæanum
Catha cassinoides W. & B.
Convolvulus Siculus L.
Davallia canariensis Sm.
Leucophaë Massoniana W. & B.
Linaria heterophylla Spreng.
Lobularia marginata W. & B.
Lotus lancerottensis W. & B.
Notochlæna vellea Desv.
Micromeria thymoides W. & B.
Ononis laxiflora Desf.
Petrophyes microbotrys Webb.
Picridium ligulatum Vent. var. inermis
Reseda crystallina W. & B.
Rhamnus crenulata Ait.
Scrophularia arguta Ait.
Senecio flaccidus Bolle
Wahlenbergia lobelioides DC.

Then back along the ridge, the vegetation of which looks at a little distance so like English hill-pasture, but is really so different, being composed mainly of annual herbs, which by the end of May are all brown and dead. From here the grey-green bush vegetation of the lonely valley below where our camels roamed at large was very striking. The only sign of life was supplied by the soaring vultures, buzzards and ravens.

March 17. Westward along the coast over the familiar stony desert with sparse Zollikoferia, etc. and frequent shallow barrancos, and then inland up the Gran Valle, lying westward of, and parallel to, our valley of yesterday, but in its lower part much broader and flatter. Here we encounter one of the most interesting of the Fuerteventura endemics, *Euphorbia handiensis* Burchard, the only cactoid spurge on the Canaries with the exception of *Euphorbia canariensis* L. It is found only in this valley and in the next to the westward, and in both

it is enormously abundant. But even on the almost flat bottom of the valley its range shows a curious restriction, for while it forms a continuous open shrubbery of millions of bushes on the eastern side of the median line of the valley, where runs the flat watercourse which serves as a road, not a single plant crosses this watercourse to its western side. The plant forms compact bushes of upright branches 1–3 feet high, and varies in an interesting manner from green to grey or brown, from thin-branched to thick-branched, from compact to lax, from almost spineless to densely spiny. Fastigiate specimens are frequent, some large plants being entirely fastigiate.

An abrupt climb at the head of the valley brings us to the top of the pass (about 1500 feet), and we stop to work the promising cliffs on the right. This ground is much drier than the summit cliffs 1200 feet above, since mists are seldom formed so low, but a number of the summit plants reappear here, with others of a xerophile nature.

The tilled ground here extends from about 500 to 1000 feet; below that a fringe of stony Zollikoferia desert stretches towards the sandy beach. Very little sand is blown inland, yet the influence of the sea extends for about half a mile, to an elevation of about 100 feet. *Euphorbia Paralias* L. and the curious *Zygophyllum Fontanesianum* W.B. are the first maritime plants to appear, followed by *Atriplex glauca* L., forming a band a quarter of a mile from the sea, with one or two other species. Below this only bare sand.

March 18. The difficulty of procuring water, food and shelter drives us northward again. Last night at Cofete we found the village deserted save for a few women, who were also preparing to retreat before the drought; we slept in a field and ate the last of our bread, started with reluctance—for the Handia cliff-wall is splendid ground, full of endemic plants, and not nearly fully explored—and our camels jog along the stony waste lying between the ocean and the precipice; on our right the sparse desert flora—Zollikoferia, *Lycium afrum*, great clumps of *Euphorbia canariensis*; on the left the equally sparse strand flora—Zygophyllum, *E. Paralias*, and several Chenopodiaceæ. Then a sharp climb, where the Handia ridge closes in on the sea, and in front we see again the sand-covered hills of the isthmus, 500–700 feet high. We cross the isthmus diagonally—many miles of sand. In the south *Ononis natrix* L. is very conspicuous, growing in little dense dark green stalked bushes 1–2 feet high—not a suitable plant-form, one would think, for so exposed a habitat. *E. Paralias* also here, and often nothing in sight but these two. Further north Zollikoferia comes back to its own, with its usual concomitants.

In the evening we reach again Matas Blancas, on the northern edge of the sand.

March 19. Inland, up a long mountain valley to Monte Cardon, a fine upstanding hill (about 2200 feet) whose basalt cliff-walls we examined. By a spring, guarded by a group of very tall *Phœnix canariensis* auct. we get a chara[1], of all things!—the only one seen on the Canaries. Cliffs very dry—too dry, for instance, for Semperviva, but not too inhospitable for a variety of Compositæ—*Asteriscus sericeus*, the shrubby *Sonchus acidus*, etc. Top of hill very barren. Lower slopes occupied mainly by Euphorbias.

March 20. North to Tuineje, across red and yellow deserts of stony soil. Much of the ground had been ploughed—scratched rather—and in a normal year would have borne crops, but now greenery was confined to isolated irrigated hollows. At Tuineje we returned to civilisation—a road, telephones, motor cars.

March 21. Motored 30 miles north to Oliva, up the centre of the island. Country generally not so arid—figs, pomegranates, almonds and palms about the villages, and good tillage with the aid of pumps. The dip down to sea-level at Puerto Cabras brought a return to absolute desert, but at Oliva some verdure again.

March 22. A conspicuous knobbly grey-green area lying north of the village proves to be a lava flow of comparatively recent date—black stone thickly covered with Ramalina and encrusting lichens. Flora interesting. *Asparagus pastorianus* W.B. the most conspicuous plant, forming 3-foot bushes; much *Kleinia neriifolia*, *Lycium afrum*, some Zollikoferia and *Rubia fruticosa* Jacq., a little *Nicotiana glauca*; colonies of *Euphorbia obtusifolia* Poir. and of the interesting cactiform *Caralluma Burchardii* N.E.Br.; many small herbs—Adonis, Lobularia, Ononis, Vicia, Erodium, Torilis, *Scrophularia arguta*, *Dipcadi fuscum*, a few grasses (Lamarckia, Setaria). In the afternoon to the hills south of Oliva, where there is a fair vegetation at about 1500 feet—mostly plants already seen. *Sempervivum* (*Aichryson*) *pulvinatum* Burchard is gathered, growing in cushions as dwarf and red as those of *S. arachnoideum* in the Alps: it proves to be identical with *A. pygmæum* W.B., a Lanzarote plant well known in cultivation.

Next day we embarked for Lanzarote, where, under less rigorous conditions, a more ample and varied flora prevailed, which served as a foretaste of the glorious vegetation of the more western islands.

[1] Subsequently determined by Messrs Groves and Bullock-Webster as *C. vulgaris* L.—the only previous record of a chara from the Canaries being one for *C. fragilis* Desv.

INDUCED HYDATHODES IN A NEW ZEALAND VERONICA

By H. H. ALLAN

(With 3 figures in the text)

VERONICA AMPLEXICAULIS is a species of limited range, containing several distinct microspecies. The form dealt with here is a decumbent rock-crevice shrub of somewhat xerophytic habit, occurring in the sub-alpine belt of Mt Peel, Canterbury. The leaves are rather small, simple, broadly oblong, sessile with semi-amplexicaul bases, perfectly glabrous, very coriaceous, glaucous.

A plant having been observed with densely shaded shoots carrying shortly petiolate leaves, the following experiment was carried out. A small specimen of typical habit and foliage was taken in the autumn and planted in a large pot. The pot was placed in an outer container in which a constant level of water was maintained. Most of the shoots were then removed and a lightly-shaded bell-jar placed over the plant. In a few weeks, in response to the changed conditions—excessive soil moisture, atmospheric humidity, and shade—numerous shoots developed from near the base of the plant, bearing thin, membranous leaves with distinct petioles. The average dimensions of these were *c.* 5 mm. wide, by *c.* 14 mm. long, with petioles *c.* 5 mm. long. The original leaves averaged *c.* 8·5–9 mm. by 14–15 mm. The thickness was reduced from *c.* 0·6 mm. to *c.* 0·2 mm. The chlorophyll tissue became rather more open, the leaves being a very pale green.

The most striking effect, however, was the development of numerous capitate hairs on the surface of the leaves. A representative example (Fig. 3) had 125 hairs on the lower, and comparable numbers on the upper surface. An examination of numerous leaves on plants in the field, and of others cultivated under normal conditions, failed to show the presence of any of these capitate hairs.

The idea that the hairs serve as hydathodes was confirmed by the fact that guttation could be produced by the usual methods of suddenly checking transpiration, and of forcing water through the stem, the water studding the leaves in minute drops. This result could not be obtained with normal leaves.

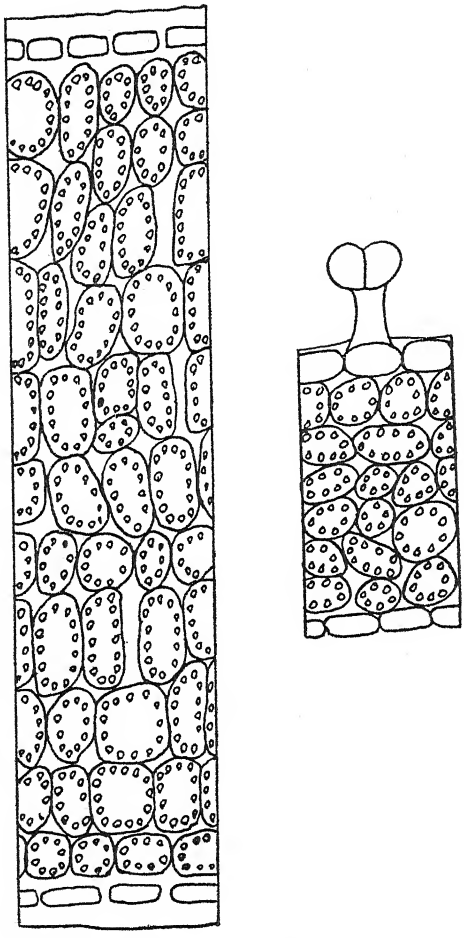

Fig. 1. T.S. of normal leaf of *Veronica amplexicaulis*. ×*c.*200.
Fig. 2. T.S. of induced leaf, showing capitate hair on upper surface. ×*c.* 200.

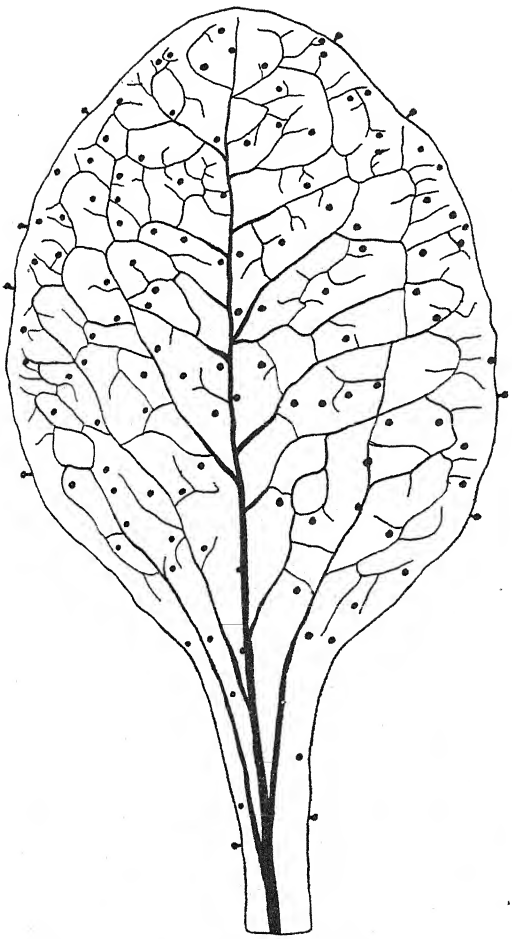

Fig. 3. Camera lucida sketch of induced leaf, showing location of capitate hairs (black dots) and portion of petiole. ×*c.* 6.

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AN ANATOMICAL AND PHYSIOLOGICAL STUDY OF THE PETIOLE IN CERTAIN SPECIES OF *POPULUS*

BY W. LEACH, M.Sc.

(The Botanical Department, University of Birmingham)

(With Plate III and 3 figures in the text)

INTRODUCTION

THE anatomical structure of the foliar organs of the various species of the genus *Populus* has received the attention of a number of botanists at different times. In practically every case, however, this structure has been investigated either from the systematic or from the comparative point of view. For instance, Col (1) briefly describes the course of the vascular strands in the leaf of *Populus alba* in a paper which deals in a comparative way with a number of families of Dicotyledons, and in this paper he mentions work of a somewhat similar nature by Petit (2). Again, Morvillez (3), dealing exclusively with the Salicaceae, has worked out the gross vascular anatomy of the petiole in a number of species of *Populus* and *Salix*, and in his paper he quotes and comments on similar work by Mlle. Komaroff (4). A paper by Graf (5) deals with the anatomy of poplar leaves mainly from the systematic standpoint. This investigator does not touch upon the vascular anatomy, but describes to some extent the structural differences presented by the lamina in the different species examined.

A piece of work which has a distinct bearing upon the present investigation is that by Ursprung on "Die physikalischen Eigenschaften der Laubblätter" (6), in which a number of experiments on leaves of various plants, including species of *Populus*, are described. From our point of view, the most important of Ursprung's experiments are those which deal with the torsional strength of petioles,

and with the behaviour of leaves when subjected to air currents of various velocities.

The present work was undertaken with a view to throwing additional light upon some of the possible physiological effects of the obviously highly specialised type of petiole possessed by many of the poplars. In connexion with this we have before us the interesting fact that, in the case of those species of *Populus* which have markedly long, slender, vertically flattened petioles, *e.g. P. tremula* and *P. canadensis*, the laminae are frequently orientated in the profile position, *i.e.* in the vertical plane. A possible physiological significance of such a state of affairs suggests itself when one remembers that in countries with hot, dry summers, the leaves of many trees assume the profile position, and that, according to the generally accepted view (7), this leaf position results in a reduction of the rate of transpiration[1].

THE ANATOMY OF THE PETIOLE

The general form and structure of the typical poplar petiole is well known, and in the already mentioned paper by Morvillez (3) will be found a detailed description of the arrangement of the vascular strands in a number of different species. We shall here confine our attention, in the main, to the physiological significance of the arrangement of the tissues in the petioles of a few of the more typical flat-petioled species.

It will be of interest at this point to examine Fig. 1, which shows series of equidistant transverse sections through the petioles of three poplars. In this figure only those tissues which have a definite mechanical function are shown, the xylem here being considered as a mechanical tissue.

The importance of the distribution of these strands in connexion with the ability of the petiole to allow of—or to withstand—bending and torsion has been pointed out by Ursprung (6). He has shown that the markedly flattened petioles of *Populus tremula*, *P. pyramidalis* and *P. nigra*, which have, in their flattened portions, a corresponding arrangement of the vascular strands in the vertical plane, can be twisted through a very large angle without injury. On the other hand, petioles which have a cylindrical arrangement of vascular and mechanical strands resist torsion to a far greater degree. In other words, perhaps the most important conclusion

[1] It is intended to carry out later a critical investigation in connexion with this point.

arrived at by Ursprung, so far as the present work is concerned, is that in the case of those species of *Populus* which have well-developed,

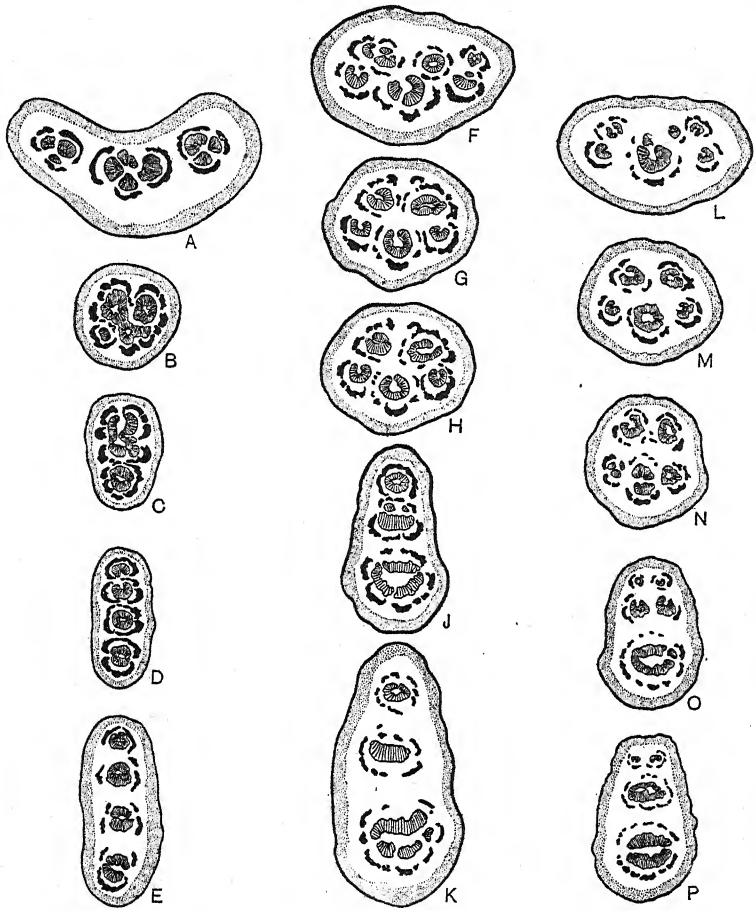

Fig. 1. Consecutive series of transverse sections cut at equal intervals, from base to apex, through the petioles of *Populus tremula* (A to E), *P. canescens* (F to K) and *P. alba* (L to P). The uppermost sections (A, F and L) are from the bases of the petioles. The adaxial sides of the sections are towards the top of the page. Collenchyma is indicated by dots, sclerenchyma by solid black, and xylem by line shading. Soft tissues are left white. × 12.

long, slender, flattened petioles, these latter organs are constructed in such a way that they offer very little resistance to torsional strains, and consequently are extremely flexible.

If Fig. 1 is examined from this point of view it will be seen that the arrangement of the vascular and other mechanical tissues in the poplar petiole is strongly reminiscent of the stems of certain lianes (8). Instead of a more or less compact ring of strengthening elements, the xylem and sclerenchyma are broken up into a number of slender strands embedded in the softer living tissues of the petiole.

The result of this structure is shown to a marked degree in the behaviour of the leaves of *Populus tremula* with regard to wind.

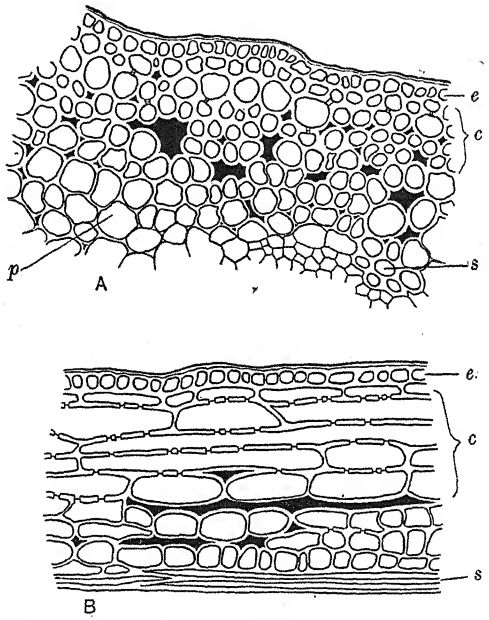

Fig. 2. A. Transverse section through the lateral tissues in the flattened region of the petiole of *Populus tremula*. B. Longitudinal section through same. *e*, epidermis; *c*, collenchyma; *s*, sclerenchyma; *p*, thin walled cells (parenchyma). Intercellular spaces marked in solid black. × 160.

Even in a light breeze the leaves of this tree may be observed to be in a state of perpetual rotation and oscillation which must cause twisting and transverse movements of the mechanical strands of the petiole in relation to one another. These movements must occasion corresponding movement of the soft living tissues between the strands, and these living tissues must remain functional all the time. We therefore expect to find these soft tissues exhibiting features which may be related to the more or less continual movement and distortion to which they are subjected.

In connexion with this point it is of interest to examine Fig. 2, which shows longitudinal and transverse sections through a portion of the lateral tissue in the flattened region of the petiole of *P. tremula*. A noticeable feature is the presence of large, longitudinally elongated intercellular spaces (marked in black), distributed mainly towards the inner margin of the collenchymatous sheath.

These large intercellular spaces are not peculiar to the genus *Populus*, but are found in other petioles which undergo strong flexure by the wind, *e.g.* in *Betula alba*, a species which like *P. tremula* has very mobile leaves; they are especially well marked, while, on the other hand, they are absent from the petiole of *Aesculus hippocastanum*, which is of a very rigid construction.

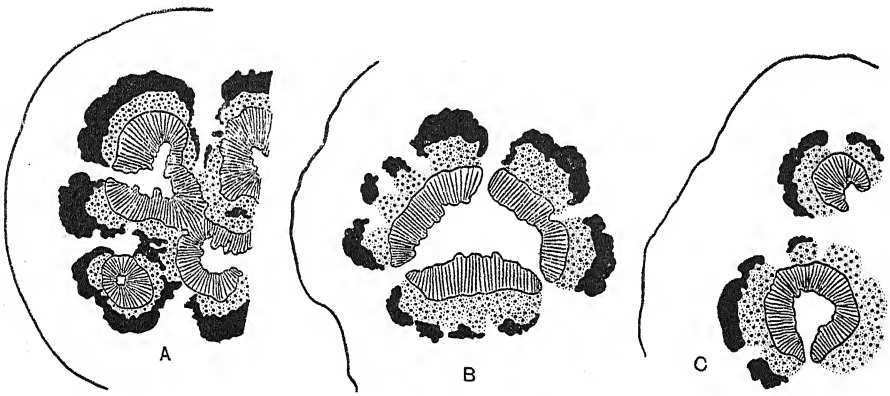

Fig. 3. Transverse sections through portions of the petioles of *Populus tremula* (A), *P. canescens* (B) and *P. alba* (C), showing the relation between xylem (indicated by line shading), phloem (dotted) and sclerenchyma (solid black). The heavy line indicates the position of the epidermis. All × 50.

It will be easily understood that the presence of these large intercellular spaces will, when the leaves are thrown into oscillation by the wind, have the effect of allowing the living cells of the ground tissue to move in relation to one another without suffering damage[1].

Now to consider the phloem, which is a delicate, easily damaged tissue, and one in which, normally, intercellular spaces do not occur; we find that in the case of those poplars which have markedly flexible petioles, *e.g. P. tremula*, the strands of fibres surrounding the vascular strands are of such a cross section that they form channels running longitudinally along the outside of the xylem. In

[1] It is possible that these intercellular spaces may owe their origin to a forcible separation of the cells in the young petiole by this frequent oscillation.

these channels lie the phloem groups, shown in Fig. 3 *A*, which is a drawing made under a higher magnification than that used for Fig. 1. Thus the phloem is more or less completely protected against pressure in this petiole, being virtually in tubes of mechanical tissue.

On the other hand, when one examines the petiole of *P. alba*, which is comparatively rigid, and supports a relatively small lamina, the sclerenchymatous strands do not offer a similar amount of protection, as will be seen in Fig. 3 *C*; again, *P. canescens*, which is in many ways intermediate in the structure of its leaves between *P. tremula* and *P. alba*, has petioles which are correspondingly intermediate in structure when considered in relation to phloem protection (see Fig. 3 *B*).

In connexion with the flexibility of the petioles of the different species of *Populus*, it becomes obvious if leafy twigs which have been allowed to wilt are examined, that the turgor of the parenchymatous ground tissue cells has an important mechanical function, in the direction of giving the requisite amount of rigidity to the organs, under normal growth conditions. As will be seen in Fig. 1, the ground tissue is enclosed in a subepidermal collenchymatous sheath which prevents its expansion in a transverse direction when its cells become turgid, consequently the tendency of the turgid cells to expand causes them to exert a stretching force on the longitudinal mechanical strands of the petiole and so stiffen it.

PHYSIOLOGICAL RELATIONS BETWEEN PETIOLE AND LAMINA

In order to gain a knowledge of the actual conditions as regards turgor, existing in the petioles of the various species under observation, and of the results arising from changes in these conditions, a number of experiments were performed.

As a starting-point we have the fact that the majority of leaves, when caused to wilt, droop into the profile position. In order to ascertain whether the leaves of species of *Populus*, having the long, laterally compressed type of petiole, were particularly sensitive in this way to the effects of water shortage, a number of experiments were performed. On three different days, leafy twigs of approximately corresponding size were cut from a number of trees, including three species of *Populus*, and brought into the laboratory. They were quickly clamped in their natural positions, and the periods of time required for all the leaves of each twig to assume the profile

position were recorded. The results of the observations are given below[1]:

	Exp. 1	Exp. 2	Exp. 3	Average
Populus nigra ...	80 min.	35 min.	50 min.	55 min.
Populus canadensis ...	30 ,,	42 ,,	25 ,,	32 ,,
Populus canescens ...	30 ,,	63 ,,	60 ,,	51 ,,
Prunus cerasus ...	60 ,,	52 ,,	70 ,,	61 ,,
Fagus sylvatica ...	65 ,,	85 ,,	70 ,,	73 ,,
Betula alba	40 ,,	43 ,,	50 ,,	44 ,,
Polygonum cuspidatum	60 ,,	55 ,,	35 ,,	50 ,,

It will be seen from the above times that the behaviour of leaves under wilting conditions is very variable, and although only three experiments were performed, the results were sufficiently irregular to indicate that the species of *Populus* examined showed no marked advantage over the rest in the direction of rapid assumption of the profile position. This fact, however, does not preclude the possibility that the profile position of the leaves may have a distinctly beneficial effect upon plants during drought, in the direction of reducing the transpiration rate, and consequently the danger of desiccation.

In connexion with the above observations, a matter of considerable interest is the way in which the profile position is brought about. In the case of the leaves of *Prunus*, *Fagus*, *Betula* and *Polygonum*, the assumption of the profile position is the result of a simple drooping of the petiole, due to the combined effect of gravity and loss of turgor, whereas in the case of species of *Populus* the movement of the leaves during wilting involves three types of motion, viz.: (*a*) a downward bending of the petiole, the curvature mainly taking place near the base of that organ; (*b*) a rotation of the lamina due to twisting of the petiole; and (*c*) a sharp bending of the petiole in its flattened region. These motions are the combined effect of reduction in turgor of the ground tissue cells of the petiole (see water-content estimation described later) and the force of gravity. With regard to the torsion of the petiole and the rotation of the lamina, this was found to be a gravitational effect resulting from slight asymmetry of the latter organ. Pl. III, figs. 1 to 6, show the effect of these three motions during wilting in twigs of *P. nigra* and *P. tremula*. In order to obtain Figs. 1 to 4 (Pl. III)[2], twigs were cut (their cut ends being placed in test-tubes of water), clamped in

[1] Observations were only made on those leaves which were in the horizontal position at the beginning of the experiment.

[2] Pl. III, figs. 5 and 6, are from a negative kindly lent by Prof. R. H. Yapp. They show side views of twigs of *P. tremula* in the first and final stages of the movement of the leaves into the profile position during wilting.

their natural positions, and photographed from above against a white background; the test-tubes were then removed and after a short time, during which wilting took place, further exposures were made. These photographs, especially the two of the *P. tremula* twig (Pl. III, figs. 3 and 4), show in a striking way the change in the amount of leaf surface, exposed to the direct rays of the sun, which results from the assumption of this position.

It will be of interest to consider the results obtained from a number of water-content estimations, which were carried out upon the laminae and petioles of various leaves. These experiments were carried out by the usual method, the petioles and laminae being cut from the twigs with a safety razor blade and placed immediately into dry weighing bottles which were always cooled in a desiccator before weighing. The figures given in columns 2 and 3 of the following tables and used for comparison purposes are the water-contents (w.c.) of the organs expressed as percentages of their dry weights.

Estimations were first carried out upon the leaves of *Populus nigra*, *P. canadensis*, *P. canescens* and *P. tremula*. The water-contents of the petioles and laminae were found of normal, fully turgid leaves, orientated in the horizontal position. (In the case of *P. canadensis*, a species which normally has many of its leaves hanging in a more or less profile position, only leaves with horizontal laminae were used.) Other leafy twigs of the same species were placed in the laboratory and allowed to wilt, and the petioles and laminae were transferred to weighing bottles, for water-content determination, as soon as the laminae had taken up the profile position. The results of these estimations are shown in Tables I to VIII.

(A) *Populus nigra.*

TABLE I. Turgid leaves (horizontal position)

No. of Exp.	w.c. of petiole = Pt	w.c. of lamina = Lt	Lt/Pt
1	268·4	219·2	0·82
2	278·3	229·4	0·82
3	376·8	350·4	0·93
4	317·5	294·7	0·92
5	325·6	247·2	0·76
6	383·5	313·1	0·82
Average	Pt′ = 325·0	Lt′ = 275·7	0·85

TABLE II. Flaccid leaves (profile position)

No. of Exp.	w.c. of petiole = Pf	w.c. of lamina = Lf	Lf/Pf
7	179·8	210·1	1·11
8	181·6	184·8	1·02
9	174·8	197·0	1·13
10	192·1	187·9	0·98
11	173·5	187·1	1·08
12	180·2	177·8	0·99
Average	Pf′ = 180·3	Lf′ = 190·8	1·05

Pt′/Pf′ = 1·80.
Lt′/Lf′ = 1·45.

(B) *Populus canadensis.*

TABLE III. Turgid leaves (horizontal position)

No. of Exp.	w.c. of petiole = Pt	w.c. of lamina = Lt	Lt/Pt
13	363·1	321·4	0·89
14	401·5	329·3	0·82
15	435·7	343·0	0·79
16	382·7	324·1	0·85
17	420·0	361·9	0·81
Average	Pt′ = 400·6	Lt′ = 335·9	0·83

TABLE IV. Flaccid leaves (profile position)

No. of Exp.	w.c. of petiole = Pf	w.c. of lamina = Lf	Lf/Pf
18	228·3	213·6	0·94
19	214·7	210·4	0·98
20	208·9	202·4	0·97
21	197·7	200·0	1·01
22	200·0	204·9	1·02
Average	Pf′ = 209·9	Lf′ = 206·3	0·98

Pt′/Pf′ = 1·91.
Lt′/Lf′ = 1·14.

(C) *Populus canescens.*

TABLE V. Turgid leaves (horizontal position)

No. of Exp.	w.c. of petiole = Pt	w.c. of lamina = Lt	Lt/Pt
23	263·3	167·5	0·64
24	262·3	177·8	0·68
25	296·9	186·3	0·63
26	269·0	167·8	0·62
27	279·5	177·5	0·65
28	254·1	168·7	0·67
Average	Pt′ = 270·9	Lt′ = 174·3	0·65

TABLE VI. Flaccid leaves (profile position)

No. of Exp.	w.c. of petiole = Pf	w.c. of lamina = Lf	Lf/Pf
29	237·1	163·5	0·70
30	217·7	156·9	0·72
31	220·3	163·2	0·74
32	215·1	157·1	0·73
33	218·8	161·6	0·74
34	212·5	152·6	0·72
Average	Pf′ = 220·3	Lf′ = 159·2	0·73

Pt′/Pf′ = 1·23.
Lt′/Lf′ = 1·09.

(D) *Populus tremula.*

TABLE VII. Turgid leaves (horizontal position)

No. of Exp.	w.c. of petiole = Pt	w.c. of lamina = Lt	Lt/Pt
35	203·0	136·4	0·67
36	206·5	136·8	0·66
37	197·1	138·1	0·70
38	203·7	149·4	0·73
39	180·0	120·8	0·66
40	196·5	140·8	0·72
Average	Pt′ = 197·8	Lt′ = 137·1	0·69

TABLE VIII. Flaccid leaves (profile position)

No. of Exp.	w.c. of petiole = Pf	w.c. of lamina = Lf	Lf/Pf
41	186·6	130·7	0·70
42	174·6	131·7	0·75
43	157·0	129·7	0·84
44	161·0	133·6	0·83
45	125·0	137·0	1·10
46	145·5	117·8	0·81
Average	Pf′ = 158·3	Lf′ = 130·1	0·84

Pt′/Pf′ = 1·25.
Lt′/Lf′ = 1·05.

In the above tables various ratios are used for the purpose of carrying out comparisons, this probably being the simplest way of dealing with the figures involved. If the quantities representing the ratios of the water-contents of the laminae to those of the petioles are examined, first in the case of the turgid leaves (Lt/Pt) of each of the four species of *Populus* experimented upon, and then in the case of the flaccid leaves (Lf/Pf), it will be seen that for each species the ratio Lf/Pf has a higher value than the ratio Lt/Pt. The interpretation of this fact is, that during wilting, or during the movement of a leaf from the horizontal to the profile position, the percentage reduction of water-content is greater in the case of the petiole than in the case of the lamina.

A comparison of the average ratios Pt′/Pf′ and Lt′/Lf′ for each species leads us to a similar conclusion, as it will be seen that the ratio Pt′/Pf′ is always greater than the ratio Lt′/Lf′ for each of the three species examined.

In order to ascertain whether this state of affairs is peculiar to these few species of *Populus*, a number of similar water-content estimations were made, using leaves of *Prunus cerasus* and *Polygonum cuspidatum*. These results are given in Tables IX to XII.

(E) *Prunus cerasus.*

TABLE IX. Turgid leaves (horizontal position)

No. of Exp.	w.c. of petiole = Pt	w.c. of lamina = Lt	Lt/Pt
47	261·4	183·5	0·70
48	271·9	191·8	0·71
49	261·0	198·6	0·76
Average	Pt′ = 264·8	Lt′ = 191·3	0·72

TABLE X. Flaccid leaves (profile position)

No. of Exp.	w.c. of petiole = Pf	w.c. of lamina = Lf	Lf/Pf
50	237·1	166·3	0·70
51	202·7	170·6	0·84
52	207·2	160·1	0·77
Average	Pf′ = 215·6	Lf′ = 165·7	0·77

Pt′/Pf′ = 1·23.
Lt′/Lf′ = 1·15.

(F) *Polygonum cuspidatum.*

TABLE XI. Turgid leaves (horizontal position)

No. of Exp.	w.c. of petiole = Pt	w.c. of lamina = Lt	Lt/Pt
53	477·5	316·4	0·66
54	570·5	333·1	0·58
55	509·7	300·3	0·59
Average	Pt′ = 519·2	Lt′ = 316·6	0·61

TABLE XII. Flaccid leaves (profile position)

No. of Exp.	w.c. of petiole = Pf	w.c. of lamina = Lf	Lf/Pf
56	263·1	209·5	0·80
57	219·9	205·9	0·93
58	263·3	207·2	0·79
Average	Pf′ = 248·8	Lf′ = 207·5	0·84

Pt′/Pf′ = 2·09.
Lt′/Lf′ = 1·50.

We see from the above figures that the interesting water-content relation between petiole and lamina, which we have found to exist

in the leaves of certain species of *Populus* when placed under wilting conditions, is also shown by the leaves of *Prunus cerasus* and *Polygonum cuspidatum*.

During wilting then, in the case of the species examined, the loss of water is relatively greater in the petiole than in the lamina. This seems to point to a greater loss of turgor in the cells of the petiole than in the cells of the lamina, a state of affairs which may prove to be of great interest if the assumption of the profile position by leaves can be shown to be an advantage under drought conditions; an attempt to obtain definite results upon this point will be carried out at some future time as has already been mentioned. If such is the case, we have the significant possibility before us, that, at the onset of unfavourable conditions such as water shortage, or excessive transpiration due to wind or strong insolation, a relatively greater loss of turgor occurs in the petiole than in the lamina, the result being that the leaf falls into the profile position, while, at the same time, the cells of the lamina remain sufficiently turgid to enable them to function with reasonable efficiency. A further point of some importance is the fact that stomata are absent from the epidermis of the petiole so that the water lost by that organ is probably passed on to the lamina. Here it will be of interest to refer again to Fig. 2 B, it will be seen that the cells of the collenchyma have in their thickened tangential walls numerous pits, the effect of which will be to facilitate the passage of water either in a centrifugal or a centripetal direction. When one considers that it is the turgor of the more peripheral cells which has the greatest effect in supplying rigidity to the petiole, the importance of these pits, which will allow of rapid changes in the turgor of these peripheral cells to take place, becomes obvious[1].

It will at this point be of interest briefly to outline the conclusions arrived at by Ursprung (6) in connexion with the behaviour, with regard to wind, of those poplar leaves which have long flattened petioles. He takes up an adaptational standpoint and sees, in the long flexible petiole, a structure which, by allowing the leaf to stream out before the wind, reduces the area of the lamina which is opposed to the force of the air current (Angriffsfläche); he considers that in this way the leaf is protected from mechanical damage[2]. Further protection, he points out, is afforded by the fact that, when

[1] The division of the xylem of the poplar petiole into a number of separate strands will also facilitate translocation of water, cf. Bower (11 and 12) and Wardlaw (13).

[2] See also Hansgirg (9), 1903, p. 123.

subjected to winds of high velocity, the leaves cease to be in the state of rapid motion which is caused by lighter winds, and lie one upon another in comparatively rigid groups. Thus, Ursprung's conclusions are based upon the flexibility of the petiole. If one now considers these conclusions in the light of the information gained from the above water-content estimations, it will be seen, in the first place, that the reduction in water-content of the petioles during wilting results in increased flexibility. The effect of this, in the experiments conducted in the laboratory (*i.e.* in still air), is to bring the leaves into the profile position. On the other hand, this increase in petiolar flexibility will, when the leaves are on the tree, and under the influence of wind, tend to facilitate the behaviour observed by Ursprung.

We can therefore also see the possibility of the already discussed relation between the turgor of the petiole and the turgor of the lamina having an effect in another direction. It is a well-known fact that the effect of wind upon leaves is to increase transpiration. We can thus understand that if one of the species of *Populus* considered in this paper (especially the more rigid petioled ones, *e.g.* *P. alba* and *P. canescens*) were subjected to certain wind conditions, rapid loss of water by transpiration might result. This would bring about an increase in flexibility which would more readily allow the leaves to undergo what we may term the "Ursprung effect," *i.e.* in a wind of moderate velocity they would more easily set themselves with their laminae parallel to the direction of the wind current, and so reduce the friction between the air and the surface of the lamina. The effect of this might be to reduce evaporation, although it has been suggested that a current of air flowing parallel to the stomata-bearing surface of a leaf might increase transpiration by causing the formation of a partial vacuum at the stomatal openings[1]. However, there is little doubt that in a high wind the super-position of the leaves as described by Ursprung would, besides having a protective effect against mechanical damage, tend to reduce transpiration by sheltering the stomata.

Conclusion

From a consideration of the results outlined above it seems probable that the physiological relations which exist between petiole and lamina, at any rate in the case of the species dealt with, may be of considerable biological importance. The flattened type of petiole possessed by many poplars is an obviously highly-specialised

[1] Yapp (10), 1912, p. 851.

structure and, without in any way adopting an adaptational standpoint, one is forced to recognise the fact that a close correlation exists between the anatomical construction of these organs and the habitat conditions under which the plants which bear them grow. Again, when considering the water-content relations between petiole and lamina, one finds conditions existing which suggest that the petiole may possess important physiological functions besides the one generally assigned to that organ, viz. the displaying of the lamina in the most favourable position for the reception of light, and that a definite motor function is not confined to those petioles which possess well-defined pulvini.

SUMMARY

1. The present investigation is an attempt to throw additional light upon the physiological effects arising from the highly specialised type of petiolar structure exhibited by the leaves of certain species of *Populus*.

2. The anatomical structure of the petiole is directly related to the amount of bending and torsion it is liable to undergo under natural conditions.

3. The arrangement of the different tissues in the petiole is such as will afford effective protection of the more delicate and vital ones when the organ is subject to such torsions and flexures.

4. The leaves of the species of *Populus* examined, when placed under wilting conditions, fall into the profile position, but they do not on the whole appear to do this more readily than those of some other trees experimented upon.

5. In the case of the leaves of those poplars having laterally compressed petioles, the falling of the lamina into the profile position involves both bending and torsion of the petiole.

6. Water-content estimations show a greater percentage loss of water from the petiole than from the lamina in the case of the *Populus*, and other leaves examined. This condition will, in a wind, tend to aid the effects described by Ursprung (6), or in still air will favour the rapid assumption by the leaves of the profile position. Either of these effects will probably tend to reduce excessive transpiration from the leaves in times of drought.

Before concluding, I should like to add that I am greatly indebted to Professor R. H. Yapp for suggesting the present investigation, and for giving much helpful criticism during its progress.

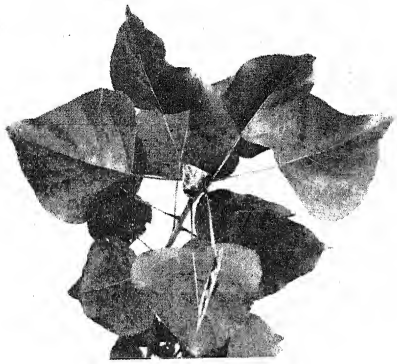

Fig. 1

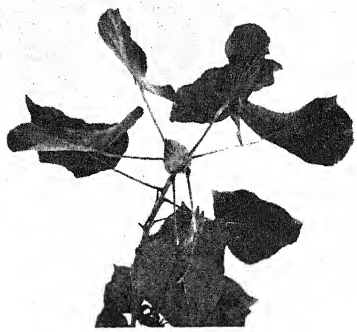

Fig. 2

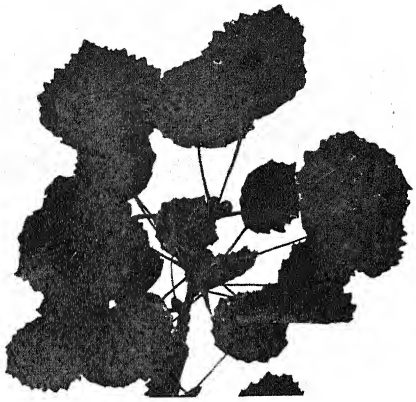

Fig. 3

Fig. 4

Fig. 5

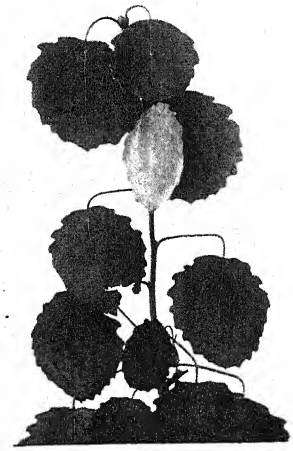

Fig. 6

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EXPLANATION OF PLATE III

Fig. 1. Twig of *Populus nigra* in turgid condition, leaves with laminae orientated horizontally. Photographed from above.

Fig. 2. The same twig as in Fig. 1, after wilting has taken place. Note that the laminae have, due to twisting of the petioles, approximately taken up the profile position.

Fig. 3. Turgid twig of *P. tremula* photographed from above.

Fig. 4. A second photograph of the twig shown in Fig. 3, after wilting. Note the marked reduction of leaf surface exposed to vertical rays of light, owing to the assumption of the profile position.

Fig. 5. Side view of a twig of *P. tremula* taken shortly after wilting has begun.

Fig. 6. Side view of another twig of *P. tremula* with leaves all wilted and completely in the profile position. This photograph shows the sharp bending which occurs in the flattened regions of the flaccid petioles.

Figs. 5 and 6. Photographs by Prof. R. H. Yapp.

THE EFFECTS OF AN ARTIFICIALLY CONTROLLED HYDRION CONCENTRATION UPON WOUND HEALING IN THE POTATO

By G. A. C. HERKLOTS

(With Plate IV and 2 figures in the text)

Introduction

To understand the development of the higher plants it is essential that the conditions governing the formation and growth of meristematic tissues should be studied. In a recent paper from this laboratory (Pearsall and Priestley (3)), attention has been drawn to the hydrogen-ion concentration of the sap bathing the meristem as possibly a factor of importance, and the present paper records the results of some experimental work designed to test the influence of this factor upon the formation and subsequent activity of a meristem. The formation of a cork phellogen in the parenchyma of a potato tuber, after the tuber has been cut, provides very suitable material and all the experiments to be described have been carried out with such material. Various varieties of potato have been employed, but in connection with the phenomena to be recorded no distinction need be drawn between the behaviour of the different varieties.

Earlier work (Priestley and Woffenden (5)–(6)) has led to the conclusion that a first stage in the process of wound healing in parenchymatous tissue is the formation of a suberin block from the products of fatty acids released from the tissue just below the cut; in this tissue starch is disappearing and fatty products appearing. Below this blocked surface, provided a nutrient sap is available, a cork phellogen subsequently appears. In the paper previously referred to (Pearsall and Priestley (3)) it is suggested that the accumulation of protoplasm within the vacuolated parenchymatous cell and its subsequent cleavage into actively growing, non-vacuolated meristematic tissue may be facilitated by the fact that the fatty acids, accumulating at the blocked surface, will produce a gradient of hydrogen-ion concentration to the normal reaction of the parenchymatous tissue of the tuber within, which will include in its range the iso-electric point of the main constituent cell proteins.

In the region where the gradient of hydrogen-ion concentration approximates to the iso-electric point, protein condensation and

synthesis may be facilitated and so the protoplasmic requirements for meristematic activity are fulfilled. The experimental investigation of this assumption is not easy, because, while the cut surface may be readily placed in contact with any desired hydrion concentration, the deposit of suberin will completely cut off this external medium from the region in which meristem formation begins. It is probable, however, that if the external solution is maintained more alkaline in reaction than the normal reaction of the suberin block (which is between *p*H 3 and *p*H 4), then the acid reaction continuously developing behind the block will not develop so rapidly or to such an extent. The result of the experiments described below is to show in no uncertain fashion that whilst the formation of the block itself, already known to depend upon oxygen, is hastened by a relatively alkaline reaction, when once the blocked surface is formed, then a relatively acid external medium facilitates the subsequent development of a meristem.

Experimental

Experiment 1

Into each of a series of sterilised dishes a 0·1 M. solution of known *p*H was poured. Three perforated china plates were placed in each dish, care being taken to avoid air bubbles. A half of a sterilised potato, seed potato of Tinwald Perfection, was placed, cut surface downwards, on each plate, the potato being immersed to the depth of $\frac{1}{4}$ inch. Finally each dish was covered with a plate of glass. At frequent intervals the *p*H of the solution was tested and if it had altered appreciably the solution was replaced by fresh. Buffer solutions A, B, C and D (see table, p. 253), with *p*H values extending from 4·6 to 8·5, were employed in the various experiments. At intervals of a week, sections were cut of one or more potatoes in each solution and stained with Sudan III and Methyl Green respectively, with a view to determining the amount of suberisation and cork meristem. Sections thus stained were mounted in glycerine jelly and kept for reference.

I. *The immediate effect of the buffer solutions on the tissues.* The effects of the acetate series fall under two heads:

(1) *p*H 4·6 and 5·0, without exception the tissues are killed rapidly; they blacken, contract and become rubber-like in nature, but remain intact.

(2) *p*H 5·5 and 5·7, no exception; killing of the tissues takes place at a slower rate and is accompanied by disintegration of the

affected parts which become rotten. In the other three series B, C, D, no immediate toxic effects are noticed. The cut surfaces of certain potatoes, however, remain white for a time, death and decay of the tissues subsequently taking place. In series B and D no potatoes decayed in solutions of *p*H 5·0 and more acid, except in one case in *p*H 5·0; and in this case the *p*H of the solution when tested was found to have become considerably more alkaline. In the most alkaline solutions the pith rots most rapidly. Thus, when death occurs in solutions with *p*H 5·5 and more alkaline, it is accompanied by disintegration of the affected tissues, but never in solutions of *p*H 5·0 or more acid. Acetates in acid buffer solutions are toxic to potato tissues.

II. *The primary reaction of the potato to the hydrogen-ion concentration.* A rapid leaching of fatty substances from the tissues of the potato into the surrounding solution takes place. This is most pronounced from *p*H 4·6 to 5·5. In these more acid examples, several layers of cells next the injured surface contain quantities of fatty materials; these are present in a mobile state, either in the form of very small globules, or as a thin film covering the walls and contents of the cells. When heated with Sudan III this film breaks up into innumerable small droplets which stain a bright brown-red. In the potatoes in solutions more alkaline than *p*H 5·5 a smaller volume of tissue contains free fat, but it is for the most part deposited on, and in, the walls of the outermost layers of cells. The tissues of the potatoes in solutions of *p*H 7·0, and more alkaline, contain a great deal of free fat in the form of large globules, but this is not observed in quantity till after suberisation has taken place.

III. *Suberisation of the cut surfaces of the healthy potatoes.* In series A (acetates), which proves toxic, no suberisation takes place.

*p*H 4·6 D	None after three weeks.	
4·8 B	Slight „ „ „	(developed after 32 days).
5·0 D	Trace „ „ „	
5·4 D	Irregular „ „	
5·4 B	Well developed after three weeks (*p*H of this solution had altered to 6·2 owing to a potato going bad).	
6·0–6·4 D	Developed after 10 days	becomes more pronounced daily.
6·0 B	Slight „ 7 „	
6·5–7·5 B	Developed „ 7 „	
7·0–8·5 D	„ „ 7 „	
8·0–8·5 C	„ „ 7 „	

The following points deserve notice:

1. At the acid end of the range about two layers of cells only are suberised, and this depth of suberisation increases in the cortical

region as the external solution becomes more alkaline, till in *p*H 8·0 and 8·5 an eighth of an inch or more is suberised. In the pith there is but little increase in the depth of suberisation.

2. Staining with Sudan III. By far the brightest red stain is obtained in the more acid cases, the colour becoming browner as the external solution becomes more alkaline till in 8·5 C and D the colour is a deep brown. *p*H 4·8 and 5·4 B after 32 days give a perfect stain.

3. The depth of suberised tissues does not increase much as the experiment progresses, although it increases considerably in intensity, *i.e.* the deposits become much heavier and give deeper staining reactions.

4. In series B, C and D after one week, although there is definite suberisation, by far the majority of fat is present as large globules, on the walls of the partially suberised cells in *p*H 8·5 and 8·0, and in less quantity in *p*H 7·5 and 7·0.

These globules are most abundant on the inner surfaces of the suberised walls, very few occurring in the living tissue within.

Another type of globule has been noticed. These globules are far larger, and have a knobby appearance, and occur either in the corners of the cells, or in the walls themselves. Far more frequently they appear to have run together, and these aggregations include within their margins the walls of several cells. Critical examination shows that these globules are the products of pectic disintegration mingled in many cases with fatty substances. When they occur within the walls themselves it is evident that the walls of two adjacent cells have come apart. In sections left in glycerine for several months, and subsequently warmed, these pectic-fat masses to a large extent come away from the walls and form large irregular blobs in the cells. These globules were first noticed in *p*H 8·0 (C), but the aggregations, which are far more frequent, are always present in the more alkaline cases (7·5–8·5), and gradually decrease in size and frequency as the solution becomes more acid. They stain a dark brown with Sudan III and a blue-green with Methyl Green. These globules have only been observed in the cortex, and the aggregations, though not confined to the cortex, are more so in the less alkaline examples. In all the alkaline cases the cells of the outer suberised layers contain and are in fact often filled with a pectic-fat mixture which stains a deep blue with Methylene Blue.

IV. *Occurrence of meristem.* The presence of meristem, correlated with pectic disintegration and fat mobility after three weeks, is shown

in Table I. No meristem has ever been observed in solutions of *p*H 8·0 or more alkaline. Figs. Nos. 1 and 2, Plate IV, illustrate the presence of a cork phellogen in *p*H 6·0 (B) and its absence in *p*H 8·5 (C).

TABLE I. Potatoes with buffer solutions, examined after 3 weeks.

*p*H	Pectic disintegration	Fats	Suberisation	Block	Meristem
4·6 D	No (No 4·6 A)	Most mobile	No	No	No
4·8 B*	No	—	Slight	Slight	Traces
5·0 D	No (5·0 A)	—	Trace	No	No
5·4 D	Yes (Yes 5·5 A	—	Irregular	,,	,,
5·4 B†	Yes	—	Good	Good	Vigorous
6·0–7·5 B	,,	Less mobile	,,	,,	,,
6·0–7·5 D	,,	—	,,	,,	6·5 ,,
8·0–8·5 C	,,	Large globules	,,	,,	No
8·0–8·5 D	,,	,,	,,	,,	,,

The above are not averages, but the actual results observed.

* After 32 days possesses suberisation on the outer layer or two, and a fairly well developed meristem, the outer wall or cell of which is suberised.

† The *p*H of the solution had altered to 6·2. There is a universal tendency for solutions from *p*H 4·6–7·5 to become more alkaline and from 8·0–8·5 to become slightly more acid.

Experiment 2

A boring was taken out of each of a series of sterilised potatoes, to a depth of about ½ inch, by means of a cork borer, diameter ⅝ inch, and a scalpel. Each hole was filled, with the aid of a pipette, with a jelly of known *p*H (see Table III, p. 253), the jelly having been previously just melted. A little thymol or mercurous chloride was added to the jelly in its preparation to check the growth of moulds. If the jelly contracted away from the potato the holes were refilled, the old jelly being left *in situ*. Sections were cut at weekly intervals and stained, as in the previous experiment. Often the knife edge of the cork borer penetrated further than the piece of tissue removed, thus a cut is formed into which the jelly may not penetrate, so that the margin of this cut behaves differently to the rest of the wound surface.

I. *The immediate effect on the tissues.* Killing of tissues does not take place in the acetate series only, but also occurs in the other buffers employed. There is a perfect range in the toxic effects produced from *p*H 4·2–8·0 (see Fig. 1). This effect is most pronounced at the acid end of the scale and gradually lessens as the buffer jelly becomes more alkaline. From *p*H 7·0–8·0 there is no dead tissue. In the previous buffer solution experiments in no case was there any killed tissue in a potato in *p*H 5·4 B and 5·4 D, but in the case of buffer jellies *p*H 5·4 B and 5·4 D prove toxic to the

same extent as 5·5 A. Evidently in these experiments the specific hydrion concentration is the determining factor and not the anion of the buffer employed, as was concluded for the case of the buffer solution experiments. Disintegration of the dead tissue is not apparent till after several weeks. The dead tissues in *p*H 5·5 A, 5·4 B, 4·6 D, and 5·0 D are far darker than in the other hydrion concentrations.

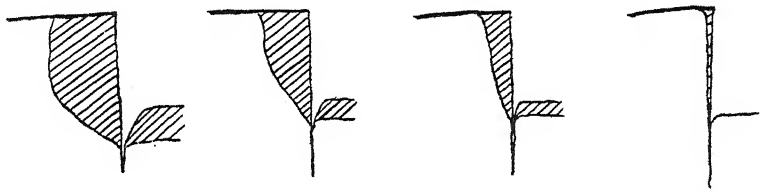

Fig. 1. The shaded portion represents the relative amount of potato tissue killed by four out of a series of eight buffer jellies, *p*H 4·2 to 8·0. Each diagram purposes to represent the half of a section through a potato from which a cylinder had been removed by means of a cork borer, the space being filled by a jelly of known *p*H.

II. *Reaction of the potato to the hydrion concentration.* In all cases the killed tissues contain considerable quantities of fatty substances.

Condition of the fats in the healthy tissues (series A, B, C and D behave similarly) *p*H 4·2–5·5. Free mobile fat present as a thin film on the walls of the cells.

*p*H 5·5–6·8. No free fat recorded, or aggregated together prior to suberisation.

*p*H 7·0–8·1. Presence of globules principally on the inner suberised walls. As in the buffer solution experiments these alkaline globules are not present in quantity till after a certain amount of suberisation has taken place.

These results agree very closely with those of the previous experiments. Leaching of fats out of the tissues into the acid buffer solutions was very pronounced, but in the present tests far more fat remains in the potato. This is only natural, as in the solution experiments a large area of wound surface was in contact with a liquid medium. The *p*H of the jellies altered a little during the three weeks of the experiment, and the acid jellies *p*H 4·6–5·0 were found to be distinctly darker than those of other *p*H. After one week acid jellies taken from the potatoes *p*H 4·6–5·5 A and *p*H 4·2–5·0 D reduced osmic acid rapidly although the acid was

reduced by no other jelly. After three weeks no jelly reduced the acid. These data suggest that the diffusion of fatty acids is immediate and that they are in the unsaturated state. Subsequently oxidation takes place, this being more rapid in the alkaline jellies.

III. *Suberisation.* In the more acid cases (roughly 4·2–5·5), where there is a considerable amount of dead tissue, after one week, suberisation is only present behind the dead tissue in the cortex and at the bottom of the cut; suberisation is complete after 2–3 weeks. In the cases where there is little or no dead tissue (6·0–8·0), after one week suberisation is continuous but thinly deposited, but increases in density daily.

*p*H 4·2 D requires four weeks to form heavy suberisation.

*p*H 4·6 forms a good deposit, but rather later than the rest.

Suberisation on the whole is heavier than in the buffer solution experiments. Internal suberisation, in the cortex, is only present to any considerable extent in *p*H 7·5 and 8·1 of the borate series. In series D, *p*H 8·0 in one or two cases has a little internal suberisation. The free fat globules and the pectic-fat aggregations which occur in series B, C and D from *p*H 7·0–8·0 are identical with those described in the buffer solution experiments and occur in the same relative positions.

IV. *Meristem.* In Part I exact figures were recorded, but in these tests, owing to a certain amount of irregularity in the early results, averages for the first three weeks are given.

One week ...	Traces in alkaline series at the bottom of the cut.		
Two weeks...	4·2–5·5 A, B, D.	Traces or nothing.	
	6·0–7·0 B	Well developed everywhere.	
	6·2–8·0 D	Well developed everywhere.	
	7·5–8·0 C	Traces only at the bottom of the cut.	
Three weeks	4·2–4·6	Nothing or poor.	
	5·0–5·5	Good—most opposite the cortex; 5·0–6·2, 2–3 cells suberised.	
	6·2–8·0 D	Good—regular; 6·8–8·0, 1 cell suberised.	
	6·0–7·0 B	Most opposite the cortex and at the bottom of the cut.	
	7·5–8·0 C	Only developed at the bottom of the cut.	
Four weeks	(Series D only.)		
	4·2	Slight traces.	
	4·6	Poor.	Cells suberised
	5·0	Continuous.	
	5·4*	Very pronounced, up to 16 cells formed; most cells opposite the jelly.	2
	6·2–6·8	Well developed.	3
	7·3–8·0	Well developed, nearly all suberised.	2–4

* See Plate IV, fig. 3.

Five weeks	(Series A, B, C.)	
	*p*H 5·5 A	Very well developed, most opposite the jelly.
	5·7 A	Well developed, most opposite the jelly.
	5·4B	Open cut; cork meristem evenly distributed.
	6·4–7·2 C	Well developed; most at the bottom of the cut.
	8·1 C	Open cut, no meristem.
	8·1 C	Closed cut, meristem only at the bottom of the cut.

In series D meristematic activity occurs in *p*H as alkaline as *p*H 8·0, although in series C no meristematic activity occurs at this *p*H, except a little at the bottom of a closed cut where the influence of the jelly is not felt. Whatever may be the reason for this difference the following generalisations can be made. Alkaline buffers check meristematic activity and increase the rate of suberisation, whereas acid buffers encourage meristematic activity but retard the subsequent suberisation.

An experiment with buffer jellies A, B and C *p*H 4·6–8·1 was carried out in an atmosphere containing 6 per cent. ethylene. The potatoes were examined weekly and refilled with jelly and replaced in a fresh ethylene air mixture. Ethylene being an unsaturated hydrocarbon renders the unsaturated fatty acids produced more mobile in the tissues (Priestley (4), Woffenden and Priestley (8)), and delays their oxidation. As a result, fats are present in the free state in all cases, although, as before, they are most mobile from *p*H 4·6–5·5 and assume a globular form in *p*H 7·0–8·1. Suberisation is delayed but not prevented, as owing to the large volume of O_2 present eventually complete oxidation of the fats takes place. After 7 days the fats diffused into the jellies are still unsaturated; however, as shown by their complete reduction of osmic acid at every *p*H, meristem formation is therefore delayed, but not prevented. After 3 weeks optimum phellogen activity is in *p*H 4·6 and 5·0. In all cases the meristem is most pronounced opposite the more acid media and in 7·5 and 8·1 C only traces are found at the bottom of the cut.

Experiment 3

Sterilised Tinwald Perfection potatoes were employed, cut in two, or one end cut off, according to size. Each half potato was set up as shown in the diagram (Fig. 2).

Molten jelly of known *p*H was poured into a flat-bottomed crystallising dish to the depth of about $\frac{1}{4}$ inch and allowed to solidify. A little more jelly was added when on the point of setting and the potato placed cut surface down on this liquid film. This rapidly

solidified. Liquid jelly was next placed, by means of a pipette, around the bottom of the potato to the height of from $\frac{1}{4}$ to $\frac{1}{2}$ inch and allowed to cool. After all the jelly had set, a just liquid, low melting point mixture of paraffin wax and vaseline was poured into the dish on top of the jelly and around the potato; thus free access of air to the cut surface of the potato was thoroughly prevented. The jelly used for these experiments contained twice as much gelatine as that used in the previous set of tests.

Two series of tests were carried out as described above. A third series, *p*H 4·4–8·0 D, was set up as above, but instead of employing the wax vaseline mixture, a greater volume of jelly was used.

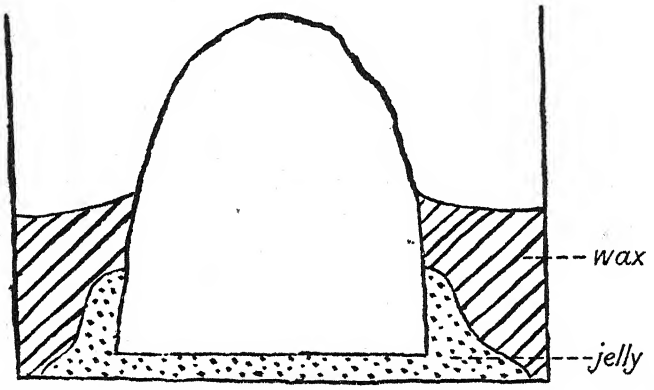

Fig. 2. A potato as set up in Exp. 3. The half of a potato was placed, cut surface down, on a buffer jelly of known *p*H (the dotted portion), this jelly, together with the adjoining part of the free surface of the potato, being finally covered with a low melting point mixture of paraffin wax and vaseline (shaded), thus excluding air from the cut surface of the tuber.

I. *The immediate effect on the tissues.* In the first two series in which the following media were employed, *p*H 4·6, 5·0, 5·5 and 5·7 A; *p*H 5·4, 6·2 and 6·8 B; 6·2, 6·4, 6·8 and 7·8 C; 7·6 and 8·0 D, the toxic effect is roughly proportional to the hydrion concentration of the jelly, most tissue being killed at *p*H 4·6 and none at 8·0. In the third series, owing to the greater depth of jelly employed, the alkaline jellies also prove toxic; the amount varies from 1 inch in *p*H 4·6 to $\frac{1}{2}$ inch at *p*H 8·0. In all cases the toxic effect is immediate, there being no increase in depth of dead tissue after the first few days.

II. *Reaction of the potato to the imposed conditions.* The absence of atmospheric oxygen has a marked effect on the tissues. In no

case out of the 30 potatoes experimented upon from *p*H 4·4–8·0 was any suberisation or meristem observed after 10 days, 35 days and 5 weeks, the respective durations of the three experiments. Hence a very definite conclusion can be drawn, that free oxygen is essential for suberisation and meristem formation. Not only was suberisation absent, but regular fatty deposits were only occasionally observed behind or in the dead tissue of the cortex. It can therefore be said that free oxygen is necessary for the deposition of or the "immobilisation" of the fats present in the healthy and dead tissues of a wounded potato. If, therefore, the mobility of the fats is unchecked, what becomes of them? All the potatoes showed large quantities of fatty substances on, not in, the walls of the cells, especially near the normal skin in the dead tissue. These fatty substances stained brown with Sudan III, but by the next morning in many of these sections mounted in glycerine they had become a bright red colour, especially in the case of the irregular fatty deposits which were evident behind the dead tissues in the cortex of certain potatoes. In no case did the jelly opposite the potato darken considerably, as in the acid examples in the presence of air in the previous experiments, but in every example recorded the jelly next the potato had become an orange-brown colour. This colour did not penetrate far into the jelly. The 6·2 B jelly next the potato, after removal from the crystallising dish and exposure to the air, rapidly went a bright orange-red. Many of the dead tissues also changed to a brighter orange colour after similar exposure. Experiment showed that some of the jellies absorbed oxygen after their removal from the dishes. In experiments 1 and 2 of this series, all the jellies were capable of reducing osmic acid, although in the final experiment less reduction was apparent.

These observations suggest that a result of the oxygen deficiency was to allow an extensive leaching of the fats out of the tissues into the jellies where they remained unsaturated.

In the last experiment an examination of the jellies showed the presence of these fats in two forms. From *p*H 5·7–8·0 a fatty substance in the form of globules of varying sizes was observed in the jelly. (See Plate IV, fig. 4.) Of this range *p*H 6·2–7·0 contained these globules in very great numbers, *p*H 5·7 and 8·0 only containing a few large ones opposite the pith. From *p*H 4·4–5·7, and again from *p*H 7·5–8·0 another phenomenon was apparent. It appeared as if the fat, instead of being present as globules, was present in a more mobile state, and had diffused straight into the jelly. Thus the surface of the

jelly, when stained with iodine, showed a perfect representation of the cut surface of the potato. In this pattern the framework did not stain with iodine and appeared light yellow, the normal jelly colour, but the spaces corresponding to the cells themselves had stained a bright reddish-brown, being darkest in colour opposite the centre of each cell. This stain was only temporary, but on restaining an hour later, an identical result was obtained.

These observations suggest that the fats are present in a mobile state (perhaps largely as unsaturated fatty acids) in *p*H 4·4–5·5; in a less mobile condition from *p*H 5·7–6·6, and again mobile from *p*H 7·5–8·0, where they are now possibly partly in the form of sodium soaps.

CONCLUSIONS

The buffer salts employed have one immediate effect upon the tissues. Employed in solutions, the acetates alone exert a toxic action in the more acid solutions, an effect which was therefore ascribed to the anion employed in the solution. On the other hand, when employed in the jellies, all solutions proved toxic at the acid end of their range, so that the effect seemed due to the hydrogen-ion concentration maintained. Up to the present this seeming contradiction has not been resolved, but this effect, which is immediate and not progressive, has not prevented a study of the subsequent suberisation and meristem formation, the processes under investigation. Another deleterious effect of the buffer solutions is the production of a certain amount of disintegration of the pectic constituents of the middle lamella. This never occurs in solutions more acid than *p*H 5·5, but is very pronounced in solutions more alkaline than *p*H 7·0.

The response of the tissue to the stimulus of wounding is to release fats in the form of unsaturated fatty acids. These are probably produced largely from carbohydrate reserves in the cells, for in many places, where fats have been observed in large quantity, starch has been absent or present only in small quantities. From *p*H 4·6 to 5·5 and again from *p*H 7·0 to 8·5, the fats are at first in a more mobile state, and it is suggested that they are present largely as unsaturated fatty acids in the acid range, and as Na or K soaps in the alkaline one. A considerable amount of evidence has accumulated to show that these fats are more quickly oxidised and hence "immobilised" in alkaline media. As a direct result, suberisation occurs most quickly from *p*H 6·4 to 8·5, especially so in the most alkaline buffers. Although regular fat deposits occur late in the more

acid series, they give a brighter stain with Sudan III than do the alkaline suberisation blocks; that obtained with *p*H 8·4, for example, being for the most part a dark, dull brown. The reason for this is that in the most alkaline experiments the fats are mixed with large quantities of pectic substances which have come from the middle lamellae. Suberisation and meristem formation are entirely dependent on the presence of free oxygen.

At first sight the optimum point of cork phellogen activity in the buffer solution experiments and that in the buffer jelly experiments seem contradictory, but they are not so in reality. In the former experiments, after 3 weeks, 6·4 appeared the most favourable *p*H and in the latter, after 4 and 5 weeks, *p*H 5·4 was found the most suitable. It must be remembered, however, that a blocked surface, and hence suberisation, is essential for meristematic activity. Now in both experiments leaching of fats took place in high hydrion concentrations, but more so in the solution experiments, as here there was a large surface exposed to the action of the buffers, whereas in the second set of tests a smaller surface was exposed, and that not to an aqueous medium but to a jelly. Suberisation was therefore late in the acid series of both experiments, but later in the solution experiments than in the jelly ones. If an acid block is favourable to meristem formation, one would expect that, as the experiment progressed in time, the point of maximum cork phellogen activity would, each week, become more acid. On a re-examination of the data it was found that not only was this supposition correct, but that the optimum points in the jelly experiments were slightly more acid than those in the buffer solution ones; this also one would expect, as the block forms more quickly in the jelly tests.

Tabulated on p. 252 are the *p*H of maximum phellogen activity at weekly intervals.

In the buffer solution experiments no meristem was observed in 8·0 C, 8·5 C, 8·0 D and 8·5 D; similarly none was present in 8·0 C buffer jelly, although a cork phellogen was well developed in 8·0 D buffer jelly. This absence of meristem may be due to (i) the tissue being too alkaline, (ii) an imperfect block, or (iii) the toxic or inhibiting effect of pectic disintegration. Nothing definite can be said in regard to these possibilities till further evidence has accumulated.

There would thus seem to be a pronounced effect produced by the external hydrion reaction upon the formation and continued activity of the meristem. Usually in the more alkaline solutions (*p*H 8 and beyond) no meristem forms at all; in the more acid

TABLE II

Buffer solutions Nos. 1 and 2.
Series 4·6, 5·0, 5·5 A; 6·0, 6·5, 7·0, 7·5 B; and 8·0 C

Time	Point of maximum meristem formation
7 days	7·0 B
14 ,,	6·5 B
21 ,,	6·5 B
33 ,,	6·0 B v. good
32 ,,	5·4 B v. good
38 ,,	4·8 B v. good

Buffer jellies, No. 4.
Series 4·6, 5·0, 5·5 A; 6·0, 6·4, 7·0 B; 7·5 and 8·1 C

Time	Point of maximum meristem formation
7 days	Nil
14 ,,	7·0
21 ,,	6·4 and 5·0

Buffer jellies, No. 5.
Series 5·5, 5·7 A; 5·4 B; 6·4, 7·2, 8·1 C

7 days	8·1 C opp. cut
35 ,,	5·5 A and 5·4 B

Only cut at these two dates.

Series D, 4·2–8·0.

7 days	7·5

No regularity afterwards.

Series 4·6 A, 5·0 A, 6·2 C.

24 days	4·6 A

Buffer jellies in 6 % C_2H_4 No. 7.
Series as in Exp. 4, 4·6–8·1.

7 days	Nil
14 ,,	6·0
21 ,,	4·6

Series D, 4·2–8·0, No. 4.

7 days	Nil
12 ,,	6·8
21 ,,	5·4
28 ,,	5·4

solutions, after the suberin block has once formed, the more acid the solution employed, the more active the phellogen tends to be, within the limits employed in these experiments, provided the experiment is continued long enough. It must be emphasised that in all cases the actual reaction just within the suberin block appears to be more acid than any buffer solution employed, lying between *p*H 3–4, but obviously in the more alkaline solutions the natural development of this reaction, due to the release of fatty acids to the inner surface of the suberin block, is continuously being neutralised with the formation of soaps of these acids.

The principal protein of the protoplasm of the potato appears to be tuberin, with an iso-electric point of *p*H 4·4 (Pearsall and Ewing (2)). The results of these experiments seem therefore to be in accordance with the view that conditions enabling the maintenance of a hydrogen-ion gradient, which includes within its range this *p*H, are favourable to the production and continued activity of a meristem.

It would probably be unwise to attempt to generalise as yet, from laboratory experiments of this type, as to the necessary continuous formation of cork at the surface of the tuber to heal the

broken surface, which lenticel expansion or slight injury must continually be exposing when the tuber is lying in the soil. From the comparison of experiments with jellies and those with solutions, it might be suggested that dry soil conditions might prove different in this respect from wet soils, and that in the dry soil a wider range of soil reaction, extending on the acid side to *p*H 4·6, might be compatible with perfect healing. On the other hand, it may be predicted with fair safety that in very alkaline soils *p*H 7·5 and beyond, whilst suberisation will take place, meristem formation will not follow and the process of pectic disintegration, which always begins, will be liable to be followed later by a rotting of the tuber associated with the invasion of saprophytic fungi and bacteria.

In the following table are given particulars of the range of buffer solutions employed in these experiments.

TABLE III. Range of buffer solutions and jellies used in Experiments

Series A

Cole *p*H	*p*H 0·1 M. solution	*p*H 0·1 M. $\frac{1}{30}$ jelly	*p*H 0·1 M. $\frac{1}{15}$ jelly
4·6	4·6	4·6	4·6
5·0	5·0	5·0	5·0
5·5	5·5	5·5	5·5
5·7	5·7	5·7	5·7

Sodium acetate and acetic acid. Cole ((i) *loc. cit.* p. 28).

Series B

5·8	4·8	5·0	—
6·0	5·4	5·4	5·4
6·15	6·0	5·95	5·6
6·5	6·5	6·4	6·2
7·0	7·0	6·7	—
7·5	7·5	7·0	6·8
8·0	8·0	7·2	—

Potassium dihydrogen phosphate and NaOH. Cole ((i) *loc. cit.* p. 27).

Series C

7·8	7·8	6·4	6·2
8·0	8·0	7·2	6·4
8·1	8·1	7·5	6·6
8·5	8·5	8·1	7·8

Boric acid, KCl and NaOH. Cole ((i) *loc. cit.* p. 28).

Series D

*p*H 0·1 M. solution	*p*H 0·1 M. $\frac{1}{30}$ jelly	*p*H 0·1 M. $\frac{1}{15}$ jelly
3·6	4·2	4·4
4·0	4·4	4·4
4·6	4·6	—
5·0	4·8	—
5·4	5·0	5·0
6·0	5·4	5·7
6·4	6·2	6·2
7·0	6·8	6·6
7·4	7·3	7·0
8·0	7·8	7·6
8·4	7·9	7·8
9·0	8·0	—

Disodium hydrogen phosphate and citric acid. T. C. McIlvaine (7).

Series A, B, C solutions are roughly three times as concentrated as given by Cole (1); this accounts for the *p*H not agreeing.

$\frac{1}{30}$ jelly is prepared by mixing equal volumes $\frac{1}{15}$ jelly (1 part by weight gelatine in 15 parts water) and 0·2 M. buffer solution. $\frac{1}{15}$ jelly is prepared similarly by mixing together equal volumes of $\frac{1}{7·5}$ jelly and 0·2 M. solution.

SUMMARY

As the result of the study of the healing of cut potatoes in the presence of media of different hydrogen-ion concentration, the following conclusions are drawn:

1. The necessity for the presence of atmospheric oxygen for both suberisation and meristematic activity is emphasised.

2. Acetates alone in acid buffer solutions prove toxic to potato tissues.

3. All buffer jellies, regardless of salts employed, kill at acid and not at alkaline *p*H. The range of toxic effect has its maximum at *p*H 4·2 and its minimum at *p*H 7·0, after which no tissues are killed.

4. Disintegration of the pectic substances of the middle lamella never occurs with buffers more acid than *p*H 5·0.

5. The fats released by the potato are in the form of unsaturated fatty acids; they are most mobile at high hydrion concentrations, but most easily oxidised and hence immobilised at low hydrion concentrations.

6. Alkalinity (especially from *p*H 7·5) promotes suberisation but retards meristematic activity.

7. After a suberised block has been formed acidity (up to *p*H 4·6, the limit of the experiments) promotes phellogen activity, but retards its subsequent suberisation.

I wish to take this opportunity of thanking Professor J. H. Priestley, under whose supervision this work has been carried out, for his valuable advice and criticism, and especially for his help in drawing up the results in their present form.

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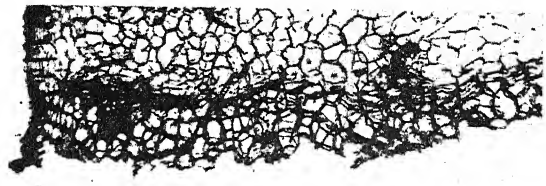

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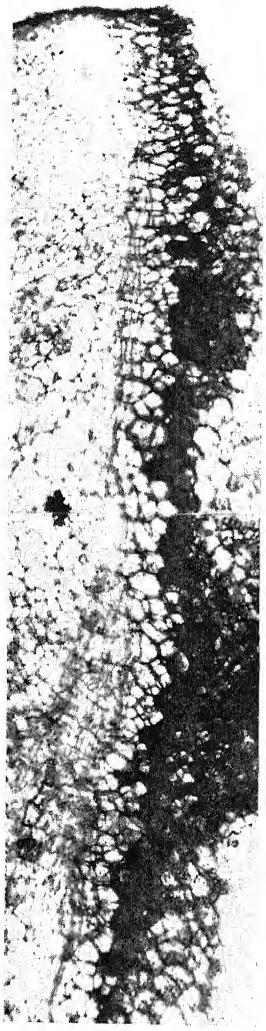

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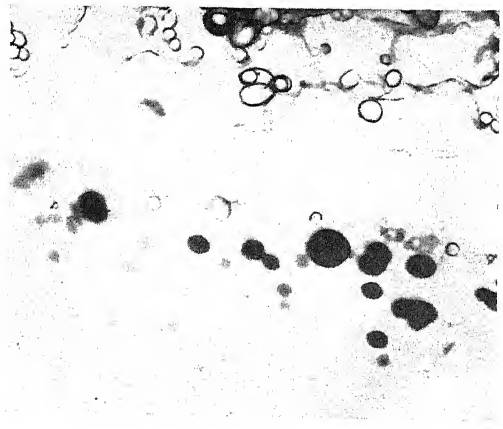

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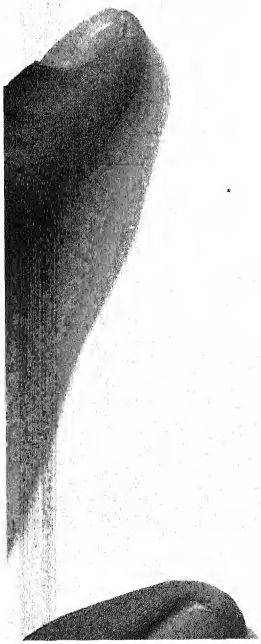
HERKLOTS—HYDRION CONCENTRATION AND WOUND HEALING IN THE POTATO



EXPLANATION OF PLATE IV

Fig. 1. Section of potato, which had been standing in a buffer solution of *p*H 6·0 B for 33 days, stained with Sudan III, showing the presence of a cork meristem within the suberised layers.

Fig. 2. Section of a potato, cut after standing in a buffer solution of *p*H 8·5 C for 33 days, stained with Sudan III, showing the total absence of meristematic activity within the suberised layers.

Fig. 3. Section through the cortex and a portion of the pith of a potato which had been under the influence of a buffer jelly *p*H 5·4 D, for 28 days. Opposite the vascular bundles as many as 17 cells, produced by phellogen activity, have been counted, of which two only had been suberised. To the right of the suberised barrier can be seen the remains of the killed tissue, most having been removed in the process of sectioning. This section was stained with Methyl Green.

Fig. 4. Section of a potato and the jelly with which it had been in contact for a period of 25 days, during which period access of air to the cut surface had been prevented. The jelly was *p*H 7·0 D. The figure shows the presence of free fat (stained with Methyl Green) in the jelly into which it had diffused from the cut surface of the tuber. Since sectioning the jelly and potato tissue have come apart slightly.

THE FIRST SUGAR OF PHOTOSYNTHESIS AND THE RÔLE OF CANE SUGAR IN THE PLANT[1]

By J. H. PRIESTLEY

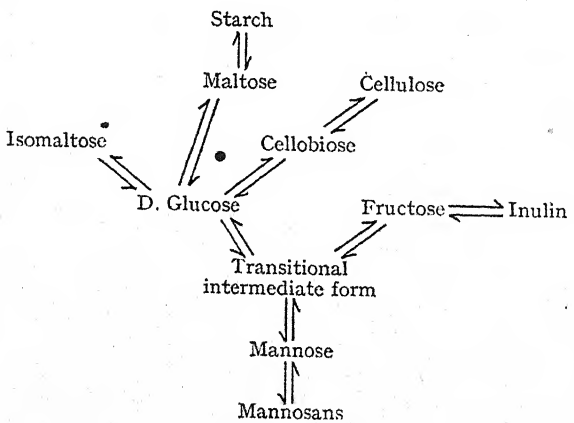
THERE are three outstanding series of investigations in the literature of plant physiology in which the problem of the first photosynthetic sugar formed in the leaf is fully discussed.

All these investigators, Brown and Morris (7), Parkin (17, 18), and Davis, Daish and Sawyer (12, 13), conclude that the evidence suggests that cane sugar plays this rôle and in general upon the same ground, viz. that if the sugars in the leaf are estimated at different times then the fluctuations in amount, with the passage of the leaf through alternating periods of light and darkness, are greater in the case of cane sugar than of the hexoses present, whilst maltose is not found in some leaves (as the snowdrop) and is perhaps only present in traces in other leaves. From the experimental data, this theoretical conclusion does not seem to the present writer to follow, because it must be remembered that in most green leaves sugars are formed

[1] This paper formed the basis of a contribution made by the author to the discussion upon Photosynthesis in Section C of the British Association in the meeting held at Saskatoon, Canada, on Friday, August 22nd, 1924.

as intermediate steps in the synthesis of more complicated bodies, such as starch, and it is a necessity of the case that for the prompt formation of these complex anhydrides the intermediate steps must be rapidly and smoothly passed through. It is to be expected then that the intermediate stages in the process, including the sugars first formed in photosynthesis, instead of accumulating in the light and therefore fluctuating in amount, should pass rapidly into other substances in the complex chain of metabolic changes so that little if any change in their concentration can be detected, and in any case no such phenomenon would occur as a local accumulation such as is characteristic of a storage product.

On examination from this standpoint the data provided by the authors cited above point to the hexoses as more probable sugars to form part of a chain of metabolic synthesis; their concentration then changing but little, as a tendency to accumulate only results in a more rapid rate of transformation into other substances. Furthermore, all the work done upon the simpler sugars in the chemical laboratory, as summarised, for instance, by E. F. Armstrong (4), points to the hexoses as in the natural path from sugar to more complex carbohydrates. In the light of the data given by the chemist (see especially Irvine (16)) it is possible to draw up a diagram of this nature as indicating the possible paths of sugar metabolism.

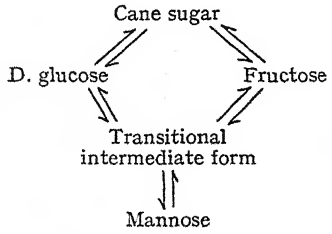


From the standpoint indicated in this diagram, the formation of any of the hexoses, d. glucose, fructose or mannose, would immediately be followed by the appearance of all three of these hexoses,

through the intermediate formation of some such substance as the "Enol" form postulated to explain the transformation of these sugars into one another in aqueous alkaline solutions ((4), *loc. cit.* p. 58).

The subsequent fate of these sugars and their consequent equilibrium concentrations, relative to one another, will depend upon the condensing system present in the neighbourhood of the photosynthetic centre, *i.e.* within the chloroplast. The series of condensations undergone by such sugars are often very complicated and associated with a system of catalysts held upon solid surfaces, as in pyrenoid and chloroplast. Under such circumstances condensations proceed in ordered series up to the formation of complex organised structures such as the starch grain. It is from this standpoint undoubtedly that we have to interpret the interesting data of Colin (9), obtained with Jerusalem artichoke shoots grafted upon sunflower root stocks or sunflower shoots upon the root stocks of the other plant. In either case starch remains strictly localised upon the sunflower side of the graft union and inulin upon the side of the Jerusalem artichoke. At the same time the root system in each case grows healthily and evidently receives adequate nourishment from the photosynthetic activity of the shoot grafted upon it. This means that soluble sugars, such as hexoses and cane sugar, can wander freely across the point of union, but these sugars come in contact in the different tissues with different condensing systems localised in the cells of these tissues, these are unable to move across the union and are unmodified by the operation of grafting, and on each side of the union these condensing systems, acting upon soluble sugar common to both plants, form in the one case starch, in the other inulin.

Cane sugar is not entered upon the diagram suggesting the possible paths of carbohydrate synthesis on the previous page. It would seem natural to add to this scheme the following additional alternative:



The condensing mechanism may possibly be invertase, although so far no successful demonstration of the reversible nature of the hydrolysis of cane sugar in the presence of this enzyme has been given. On consideration, however, there seems to the present writer to be grounds for thinking that cane sugar formation in the plant usually proceeds upon other lines and that its formation from the hexoses first formed in photosynthesis is far less direct. The grounds for this opinion will now be briefly put forward.

Weevers' (25) recent observations upon the sugars present in the leaves of variegated plants do not support the view that cane sugar is the first formed sugar in photosynthesis. In the leaves of ten out of twelve species of variegated plants the variegated patches contain saccharose alone, saccharoses and hexoses being present in the green areas. A curious point is that Weevers finds invertase also present in the variegated regions that are free from hexoses. Furthermore in *Pelargonium zonale*, in leaves kept in the dark until starch-free and placed again in the light, hexoses reappear before cane sugar.

In the xylem sap Dixon and Atkins (14) found cane sugars and hexoses usually present, maltose only very rarely. In observations made at Leeds, by Mr T. Swarbrick (22), upon the sap flowing in the sycamore, *Acer Pseudoplatanus* L., in the spring, cane sugar and hexoses were alone found in the sap. Wormall (26) has examined some 90 litres of sap collected in Leeds from cut vines under antiseptic conditions and also finds cane sugar, d. glucose and fructose the only sugars present. In the case of the sycamore it was noted that cane sugar appeared in the sycamore exudate in the spring before any hexoses at all were present and as it was accompanied by invertase the conclusion seems inevitable that in this case, at least, the hexoses arise from the cane sugar, not the cane sugar from the hexoses.

Similarly Annett (2, 3) found that in the date-palms the sap gathered under clean conditions from the wound contained less than 0·01 per cent. hexoses, the only sugar present in appreciable quantity being cane sugar. It is usually assumed that this cane sugar in the xylem exudate arises from starch—Annett, for instance, draws attention to the fact that the tissues of the palm close to the cut and bleeding surfaces are crammed with starch. When the hydrolysis of starch *in vitro* (either by diastatic enzymes or by other means) is considered, or when the recent contributions of Irvine ((16), *loc. cit.* p. 910) to our knowledge of the constitution of starch are considered, little support can be found from chemical sources for this view.

Starch on hydrolysis evidently passes through a series of changes involving continual addition of water to the molecule, but the recognisable sugars ultimately formed are the di-saccharide maltose, and then glucose. If, then, cane sugar is to be formed, it would seem necessary from the chemical standpoint to obtain first fructose from the glucose and then a synthesis of cane sugar from these two hexoses. Clearly this point of view clashes with our own observations and those of Annett as to the relative order of appearance of cane sugar and hexoses in the xylem sap.

There are however a whole series of data contributed by various workers in plant physiology which also throw doubt upon the little challenged assumption that the cane sugar in the xylem sap arises from the starch contained in tissues in or near the xylem. Dixon and Atkins (14, 5) and other workers have made it very clear that there is usually sugar moving in the sap within the xylem vessel and Dixon (15) is at present arguing that this is the main channel for the movement of carbohydrates down the plant. Only on one or two occasions, however, do these workers report maltose in the sap, and possibly the relative frequency of this occurrence is some indication of the extent to which the starch stored in the xylem parenchyma contributes to the sugar supply in the neighbouring tracheae.

Butler, Smith and Curry (8), from a study of the distribution of these substances in the tissue of the apple tree at different seasons of the year, conclude that the concentration of cane sugar is quite independent of the distribution of starch and that saccharose is primarily a reserve material, especially as the young roots, in which it is relatively abundant in the dormant period, lose it very rapidly when the buds open in spring while the one-year-old branches then become much richer in cane sugar.

Further, the long series of experiments performed by Curtis (10, 11) would receive considerable elucidation if it should appear that the sugars moving in the xylem do not arise in the main from starch stored in the parenchyma amongst the xylem. Curtis has carried out a series of ringing experiments which certainly make it difficult to believe that the usual mechanism for the removal of the starch in the xylem parenchyma is to be found in the sap stream moving in the xylem. Perhaps the most convincing experiment on this point is that in which two rings down to the cambium are made at a short distance apart from one another along a stem. Whilst subsequently the starch disappears from this stem below and above

the pair of rings, between these rings no appreciable change in the amount of starch present takes place. Curtis, on the basis of many ringing experiments, concludes that carbohydrates do not move in the xylem. His evidence really points to the fact that certain carbohydrate stores, such as those in the xylem parenchyma, or the stores used in shoot growth in certain of his experiments, do not move in the xylem. Clearly his experimental data cannot alter the fact that carbohydrate *is* present in the xylem sap and the apparent contradiction is considerably cleared up if it can be shown that the cane sugar and hexoses in the sap arise from some source which is distinct from the sources of carbohydrate supply he has had in mind during his experiments.

Another interesting and puzzling series of data are provided by Ahrns (1), whose experiments seem to show a curious and unexpected connection between the starch-content and sugar (hexoses and saccharose) content of a leaf. The general form of the reaction starch + $H_2O \rightleftharpoons$ sugar, would certainly suggest that the appearance of sugars at the expense of starch would be favoured by the presence of water, the reaction proceeding in that direction being a hydrolysis, the formation of starch from sugar, on the other hand, proceeding with the elimination of water. That such considerations do affect these carbohydrate linkages is shown beyond dispute by the long series of enzyme syntheses by Bourquelot and Bridel and their co-workers ((6), see also (19)), who have succeeded frequently in producing synthesis by using a medium in which water is largely replaced by alcohol.

Ahrns' results however establish very clearly that in the case both of leaves either growing upon or detached from the plant the formation of soluble sugar and the disappearance of starch are favoured by a falling water-content in the leaf. In the wilting leaf cane sugar and hexoses are observed to appear at the expense of starch, all the starch being thus accounted for with the exception of a small amount assumed lost in respiration. In starch-containing leaves Ahrns found the cane sugar content to increase both in dark and light with falling water-content and to fall with increasing water-content so that the sugar-content seems to be an inverse function of the water-content. These results are difficult of interpretation upon any point of view, but they are clearly not in accordance with the assumption that cane sugar arises by a simple process of hydrolysis from starch, whilst the accumulation of cane sugar indifferently in dark or light eliminates the assumption that photosynthesis is concerned.

For the reasons given above, then, it is thought that the case for the formation of cane sugar, as a primary photosynthetic sugar, or as an intermediate sugar, either in the synthesis or hydrolysis of starch, rests upon a very insecure foundation. Ahrns concludes from experiments upon the localisation of sugar in the veins and leaf stalks of *Tropaeolum* and *Helianthus* that hexoses alone function in translocation, a conclusion in accord with Ruhland's (24) earlier extensive series of experiments, and, bearing Curtis' ringing experiments in mind, it is difficult to assume that cane sugar functions as a means of carbohydrate translocation.

In snowdrop, as in many Monocotyledons, cane sugar is clearly a storage form of carbohydrate but the universal distribution of small concentrations of this carbohydrate, its constant presence in the xylem exudates examined, its appearance in leaves with fall of water-content suggest that cane sugar is playing a wider rôle in general metabolism.

In this connection a suggestion previously made in reference to the sugars producing osmotic pressure within the vascular system of the root (Priestley (21)) perhaps deserves a little fuller exploration.

In the case of exudation pressure developed in the root system, if the sap rising into the shoot is collected, cane sugar and hexoses are uniformly present. The water in this sap is entering the root system mainly, if not entirely, in an absorptive region close behind the growing point, covered with root hairs and with a cortex separated from the vascular system by a primary endodermis. In the vascular system in this region a steady osmotic pressure is apparently maintained in spite of the continued loss of sugar with the sap moving upwards in the system. Sugar is therefore continually being released into the xylem in this region. Since this point has arisen as of interest, the root system in this region has been carefully examined as occasion permits and it may be said with confidence that all the tissues of the root in this region seldom if ever contain starch and that even if starch is occasionally to be found in the cortex it does not appear within the endodermis.

Considerations discussed previously (20, 21) make it unlikely that sugar formed from starch in the cortex would make its way into the xylem elements. Therefore it may be concluded that the sugar supply contributing to maintain osmotic pressure in the xylem of the root rarely if ever is derived directly from starch and that this sugar practically always consists in great part of cane sugar. The question then arises as to the origin of this sugar.

It was suggested (Priestley(21)) that the maintenance of a supply of organic solutes was due to the loss of substance to the xylem by the cells at the end of the xylem column and clothing its sides which were in process of differentiating into xylem. Only growing roots maintain an exudation pressure, and obviously so long as growth continues this supply of solutes is available, as differentiation of new elements continues. Furthermore, clearly throughout the length of the vascular column as new elements are added to the vascular system the same source of organic solutes and of sugar will be available.

To discuss only two cases. In the Dicotyledon with a vascular cambium, elements situated between the cambium and the xylem differentiate into xylem and thus lose solutes to the xylem. These elements, from which the sugars may come, unlike the storage elements of the xylem parenchyma, *never contain starch.*

In the case of the palm shoots investigated by Annett it is true that the parenchyma in the neighbourhood of the vascular system contains much starch, but here again the actual elements differentiating into xylem and losing organic solutes in the process are derived but recently from the apical meristem and will probably be found to be free from starch. Here again, then, the connection between starch and cane sugar may prove to be indirect and in all these cases the only connection between starch and cane sugar would seem to be along the following lines:

Starch	or other carbohydrate reserve or primary photosynthetic product.	→ Used in metabolic synthesis in meristematic protoplast.	→ Cane sugar released from differentiating cell.

All these considerations have led the present writer to this conclusion, that the cane sugar widespread in plant tissues but not concentrated in amount as a storage product arises as a secondary product, formed first of all during the complicated metabolism associated with protoplasmic construction and therefore present in the growing cell. Subsequently, during the secondary events that follow in the further history of this cell, the cane sugar may be released from the protoplast and become recognisable as an individual product no longer completely masked in the intricate machinery of protoplasmic metabolism.

In this case no direct connexion may be anticipated between starch and soluble sugars and the water-content favouring hydrolysis or synthesis. The constant presence of saccharose in a growing

vascular system may be anticipated, whilst at the same time the result of Curtis' ringing experiments are not in contradiction with the fact. Further, light also seems to be thrown from this point of view upon the results of Butler, Smith and Curry upon the movement of cane sugar within the apple tree, these movements showing cane sugar as more than a storage product. Furthermore, Weevers' observation that cane sugar and invertase occur together in the variegated portion of the leaf becomes intelligible, as also the presence of cane sugar and invertase without hexoses in the xylem sap in early spring. Obviously invertase and saccharose may have no opportunity to interact in presence of water until the saccharose is released from the cell.

Whilst then this view seems to reconcile a number of outstanding facts about the occurrence of cane sugar, which remain inexplicable so long as cane sugar is interpreted as a primary sugar of photosynthesis or as a storage product arising directly from the first formed hexoses, it is recognised that much more experimental evidence is required before it can be regarded as anything more than a working hypothesis. One fact that seems to fit into place upon this view has come to light in recent work by E. Rhodes in this laboratory upon the fatty substance present in the germinating radicle of the broad bean as contrasted with the dry ungerminated radicle. In the germinating tissue the lipins extracted with alcohol are associated with sugar extremely closely and their separation from the sugar is very difficult. Upon hydrolysis the sugar proves to be composed of glucose and fructose in such proportion that quite a concentrated solution is without detectable rotation under the polariscope. This at least suggests that these hexoses are present in proportions very similar to that in which they exist in saccharose. This sugar appears in the last two millimetres of the germinating root tip, in the region where meristem cells are vacuolating and differentiating and is not present until the process of growth and differentiation has commenced after the beans have been soaked in water and germinated. The occurrence of these sugars will be the subject of further investigation.

SUMMARY

Data available as to the concentration of hexoses and the disaccharides maltose and cane sugar in the leaf in light and darkness are usually interpreted to indicate cane sugar as the primary product of photosynthesis.

Grounds are given for concluding that these data really support the formation of hexoses as the primary photosynthetic sugars. The view is shown to be more in accordance with the chemical data.

Weevers' experiments with variegated leaves point in the same direction.

At the same time the available data as to the distribution of cane sugar are not adequately explained by its rôle as a storage product.

Neither physiological nor chemical data support the assumption frequently made that cane sugar arises directly from starch.

Based mainly on a consideration of the data as to the occurrence of cane sugar and hexoses in the vascular system, grounds are given for considering that cane sugar frequently appears in such a system upon its release from the differentiating protoplast.

Upon this view, cane sugar would not be directly connected either with the synthesis or hydrolysis of starch, but starch or other carbohydrate reserves or photosynthetic products would be used in the synthetic metabolism in the meristematic protoplast, and cane sugar would appear as a secondary product in the metabolism of this cell, being subsequently occasionally released from these cells as they vacuolate and differentiate.

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THE GEOGRAPHICAL AFFINITIES OF THE FLORA OF JEBEL MARRA

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(With 2 figures in the text)

THE great desert region of N. Africa is divided into two rather unequal parts by a complex but narrow system of hills and mountains running approximately north-west and south-east. The region to the west of this dividing line is the Sahara proper, while to the east lies the Libyan desert with a slightly higher average altitude. This dividing crest of high land has no general name and consists of a number of more or less isolated mountain masses. These may be considered to fall into three main groups. The north-west group consists of the Tasili Plateau and the Ahaggar Mts, and in places reaches a height of 9000 ft. The central portion of the range is the elevated ridge known as the Tibesti, some 500 miles long and including peaks 11,000 ft. high. The south-east portion comprises the two plateaux Erdi and Ennedi, with altitudes of about 4000 ft., and the group of mountains lying to the west of El Fasher. Of these mountains the most important is the great elevated mass of the Jebel Marra. This forms, with its outliers, the south-west extremity of the whole desert chain and is known to contain peaks exceeding 10,000 ft.

The name Jebel Marra is now generally given to a large volcanic massif situated at 13° N. and 24° E. and consisting of a series of peaks rising out of a plateau of metamorphic rocks, mainly granitic gneisses. The main mass has two outliers to the north-east; Jebel Tagabo of sandstone, and Jebel Medob of volcanic origin. The latter reaches an elevation of 6000 ft. The plain surrounding Jebel Marra and its outliers is known as the Plain of Darfur.

The great crest crossing the Sahara has been but little explored, Jebel Marra itself being the only part about which much information is available. Even this material has been collected only in the last few years, notably from the accounts of Colonel Tilho, a French officer who saw service here during the late war. This officer was also the first to estimate the heights of many of the peaks with any

degree of accuracy and to show that in most cases they were very much higher than had been previously supposed. His figures are supported by the subsequent observations of Admiral Lynes.

Jebel Marra

Jebel Marra itself is an extensive volcanic massif some 55 miles long and 25 miles wide, with its longer axis running N.E. and S.W. It is divided by water courses into three portions, northern, central and south-western, with areas in the proportion 4 : 3 : 1. The northern block, although the largest and driest, is the most homogeneous, merely consisting of broken mountain masses which do not exceed a height of 7500 ft. The central block contains three important features. The first of these is the Niurnya amphitheatre floor some 4 miles by 1 mile at a level of nearly 7000 ft. It is abundantly watered and is surrounded by peaks, one of which, Jebel Niurnya, 10,100 ft., is the highest in the whole massif. The second feature is the great central plateau, 3 miles by 2 miles, at 9200 ft., among the peaks. The third feature is that of the downs, which are upland slopes at a height of from 7000 to 8000 ft. among the peaks. The south-westerly block consists almost entirely of the huge crater of an extinct volcano surrounded by a series of peaks. The floor of this crater lies at 7400 ft. and contains two lakes. The more northerly of these is salt and is the puddle of the main crater. The more southerly is almost fresh and appears to cover the bed of a secondary smaller volcanic crater within the larger. The peaks round the main crater are highest and most imposing on the S.W., where two or three reach a height of about 10,000 ft. The most south-westerly of all is the peak to which the name Jebel Marra was probably originally confined. The base of the whole massif is well defined at about 4000 ft. and the steepest side is on the east of the central portion. To the north it descends easily by long terraces to the pass which separates it from Jebel Si.

The geographical position and relations of Jebel Marra make the study of its flora and fauna of very great interest, especially from the point of view of distribution. Itself within the Tropic of Cancer and lying practically at the centre of the whole African continent, it is at the same time the most southerly extremity of a range of hills and mountains traversing the great desert region and forming a connecting link between the mountains of N. Africa and those of central tropical Africa. On the east and west, and to a lesser degree on the south, it is surrounded by comparatively low

lying plains beyond which rise the great mountains of tropical Africa.

Jebel Marra, then, is an elevated mass with a narrow connection of high ground to the north but surrounded by plains in all other directions. Beyond these plains at a distance of some hundreds of

Fig. 1. Sketch map of Africa showing the position of Jebel Marra in relation to the other mountainous regions of the continent.

miles lie the great African mountains on the arc of a vast semi-circle of which Jebel Marra is the centre.

These great mountainous regions may, for the purposes of this paper, be considered as composed of four main elements, namely:

1. The Abyssinian Mts, lying some 900 miles due east and reaching heights of over 12,000 ft.

2. The three great mountains of Elgon, 14,000 ft., Kenya,

17,000 ft., and Kilimandjaro, 19,000 ft., about 1200 miles to the south-east.

3. The mountains of Ruwenzori and the Central African Highlands, reaching heights of 16,500 ft. and situated about 1000 miles S.S.E.

4. The Cameroon Mts, with heights up to 14,000 ft. and lying 1000–1500 miles away in a south-westerly direction.

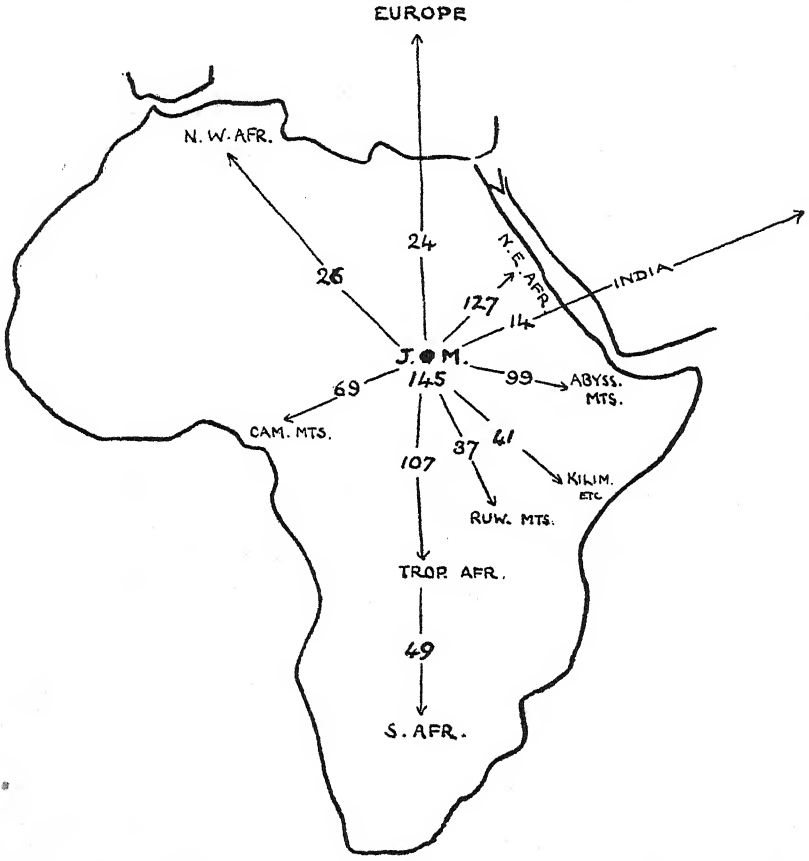

Fig. 2. Sketch map of Africa showing the relationships of the flora of Jebel Marra. The figures indicate the number of species (out of 145) common to J. M. and to the various places to which the arrows point.

The Flora

Our knowledge of the flora of Jebel Marra is derived from the two collections made by Admiral H. Lynes during his visits to

Darfur in 1920 and 1921–2. These collections were presented by the collector to the British Museum (Natural History) and lists of them have already been published. In the following pages only the Phanerogams have been considered.

The flora as at present known consists of some 145 species and varieties, included in 127 genera and representing 53 families. 22 of the species are Monocotyledons and 123 Dicotyledons. Of the latter 62 belong to the *Archichlamydeae* and 61 to the *Metachlamydeae*. These proportions compare closely with similar divisions throughout the world except that the proportion of *Metachlamydeae* is slightly above the average.

FAMILIES

Of the whole 53 families none are confined to Africa. All but three (*Burseraceae, Combretaceae* and *Pedaliaceae*, which do not reach N. Africa) range throughout the continent. No less than 37 of the families are well represented in the northern hemisphere (32 having species in Britain), while the remainder are widely spread in the tropics and sub-tropics. Three of the families, *Solanaceae, Bignoniaceae* and *Myrtaceae*, have the bulk of their species in the southern hemisphere; five are predominantly northern and 40 are generally distributed between the two. The Monocotyledons are all included in the latter.

The following table gives a list, in order of magnitude, of the families most strongly developed in the flora of Jebel Marra, compared with the order of the same families over all Africa and over the whole world:

		Order in Jebel Marra	Order in Africa	Order in the world
Compositae	19 spp.	1	1	1
Leguminosae	16 ,,	2	2	2
Scrophulariaceae	10 ,,	3	7	8
Labiatae	9 ,,	4	7	7
Gramineae	9 ,,	4	3	6
Rubiaceae	3 ,,	10	4	4
Orchidaceae	—	—	5	3
Liliaceae	4 ,,	8	6	10
Euphorbiaceae	1 ,,	25	7	5

From this it will be seen that the flora of Jebel Marra has an unusually high proportion of *Scrophulariaceae* and *Labiatae*. In the case of the former this is correlated with the geographical position midway between the two areas, North Temperate and S. Africa, in which the family is most strongly developed. In the case of the *Labiatae* the family is strongly represented in N. and

Central Africa. The great family of the *Orchidaceae* is conspicuous by its complete absence. Another important group of plants, the Gymnosperms, is also without representatives. Two families, *Rubiaceae* and *Euphorbiaceae*, are comparatively poorly represented.

This survey shows that as far as the families are concerned the flora has a distinctly northern and warm temperate facies. The great majority of the families are widely spread in and characteristic of those regions.

Genera

The flora contains 127 genera. The great majority of the genera fall into the following categories in the numbers shown.

Cosmopolitan... ...	31	Old world temperate	2
Tropics and subtropics	28	Northern hemisphere	10
Temperate	8	Africa—India ...	12
Old world cosmopolitan	5	Confined to Africa...	9
Old world tropics ...	12		

A further group may be considered as Mediterranean—that is to say, the genera centre in one or more of the regions round that sea. This group comprises *Pulicaria*, *Echinops*, *Biserula*, *Picridium*, *Cometes* and *Otostegia*. Four genera do not fall into any of these groups. One of these, *Adenostemma*, is tropical American, but has one cosmopolitan species; the other three, *Asclepias*, *Achyrocline* and *Lonchocarpus*, are distributed through America and Africa. No genus is confined to Jebel Marra, but nine are endemic to Africa (including Madagascar). These are *Aloe*, *Helminthocarpum*, *Diplolophium*, *Blaeria*, *Astrochlaena*, *Anthospermum*, *Guizotia* and *Felicia*. Seven of these have a wide range within the continent. *Aloe* is a large genus centred in S. Africa and *Helminthocarpum* is an Abyssinian monotypic genus.

An interesting feature is seen when the average size of the genera represented is considered. The average size for all the genera known, throughout the world, is about 14 species. Only some 500, or 4 per cent., have more than 10 species and only about 200, or 1·5 per cent., have more than 25 species. In the flora of Jebel Marra these two percentages are, respectively, 89 and 73 per cent., while the average size of the genera present is 150 and 80 per cent. are above the average in size. A similar but less marked effect is noticed in the case of the families. Hence the flora is mainly composed of families and genera which are widely spread and presumably near

the zenith of their development, again emphasising the unspecialised aspect of the flora.

In the following table the genera are arranged according to their size, in species, throughout the world:

Genera with over 100 spp.		55			
,, ,, 50–100 ,,		22			
,, ,, 20– 50 ,,		20			
,, ,, 10– 20 ,,		17			
,, ,, under 10 ,,	9	*i.e.*	8 spp. 1	2 spp.	2
			6 ,, 2	1 ,,	2
			5 ,, 2		

It will be seen that a very large proportion of the genera occupy at least one quarter of the world's surface. On the other hand, there are no narrowly endemic genera and only eight are confined to Africa. Seven others have narrow distributions outside the continental limits. Two features are particularly striking. First, the number of genera which are characteristic of, or most developed in the northern temperate region of the Old World and second, the group of African-Indian genera. These have a distribution from Africa to India and are most strongly developed at the two extremities of this area. There are usually one or two connecting species between the two extremes.

SPECIES

The 145 species may be divided primarily into two classes—those which are confined to the African continent and those which have a wider distribution.

The first class of African endemics contains 81 species, or 55 per cent. of the total flora. Five of these species, belonging to the genera *Crotalaria, Rhynchosia, Silene, Lobelia* and *Coleus*, are confined to Jebel Marra itself and form the narrowly endemic element of the flora. All are well marked species, but in all cases have closely related species in tropical Africa. All belong to large and widely spread genera.

For the purpose of analysing the remaining 77 species the continent may be considered as divided up into four portions: north-west (north of the Sahara), north-east (including Abyssinia), tropical and south. The species are then seen to be distributed as follows:

21 occur in the north-east division only.
11 occur in the tropical division only.
34 occur in both north-east and tropical divisions.
11 occur in north-east, tropical and south divisions.

Of the north-east species no less than 14 are purely Abyssinian. These figures reveal two important points. First, as far as African

endemics are concerned, the flora of Jebel Marra has no affinity whatever with that of N.W. Africa. Second, no less than 59, or 78 per cent., are found also in Abyssinia and 56, or 73 per cent., in tropical Africa. Third, the 11 species common to Jebel Marra and S. Africa also occur in N.E. and tropical.

The second great class of species with distributions outside the limits of the continent, or "wides" as they may be called, comprises 64 species, or 45 per cent. of the whole flora. These fall into the following groups:

Cosmopolitan species ...	8	Northern temperate species	9
Pan-tropical species ...	11	African-Indian species ...	14
Old World tropics species	11	Smaller area species ...	11

Of this last group 10 occur in one or more of the three regions, S. Europe, Asia Minor and Syria, and may thus be considered as Mediterranean. The eleventh species, *Phagnalon scalarum*, is recorded from S. Arabia. The little group of African-Indian species is of interest in recalling a phenomenon already noticed among the genera.

The distribution, within the continent, of the 64 wide species, may be analysed in the same way as was done for the endemics, with the following results:

17 species occur all over the continent.
20 species in N.E., tropical and south.
4 species in N.E., N.W. and tropical.
4 species in N.E. and N.W.
11 species in N.E. and tropical.
1 species in tropical and south.
6 species in N.E. only.
1 species in N.W. only.

The species which are spread all over the continent are also mostly cosmopolitan or very widely distributed outside. The exceptions are *Trichodesma africanum*, *Boerhaavia plumbaginea* and *Withania somnifera*. Of the six species in the N.E. only, two extend to India, one is more wide still and the other three only reach C. and W. Asia.

The wides exhibit two features of interest. First, the very small number of species which also occur in N.W. Africa. True 25 such are noted but of these 17 are found all over the continent, 4 in three divisions and only one (*Hypericum perforatum*) is, in its African distribution, confined to N.W. Africa. The second feature is the

presence of a group of characteristically north temperate species. Certain of these are among the most conspicuous elements in the flora.

In the following table the flora may now be considered as a whole:

N.W. Africa	and Jebel Marra have			26	spp.	in common	
N.E. Africa	,,	,,	,,	,,	127	,,	,,
Abyssinian Mts	,,	,,	,,	,,	101	,,	,,
Tropical Africa	,,	,,	,,	,,	107	,,	,,
S. Africa	,,	,,	,,	,,	49	,,	,,
Europe	,,	,,	,,	,,	25	,,	,, (16 British).

These figures together with the preceding analysis make it possible to indicate the main elements of which the flora is composed. These are:

1. A cosmopolitan or very widespread element.
2. An Abyssinian-Asian element.
3. A tropical African element.
4. A Mediterranean-W. Asian element.

These elements are discussed more fully below.

Altitude Zones

Admiral Lynes considers that Jebel Marra may be divided into three zones of altitude. These are: a lower zone from the base (4000 ft.) to a height of 6000 ft.; a middle zone 6000–8000 ft.; and an upper zone above 8000 ft. A certain number of the very common tropical weeds have been omitted from the following considerations. The distribution of the remainder is 64 in the upper zone, 52 in the middle zone and 22 to the lower zone.

The upper zone. 37 of the species in this zone are confined to Africa while 27 are wides. Of these latter 3 species are cosmopolitan, 3 are Old World tropical, 3 are north temperate, 9 are African-Indian and 9 are Mediterranean. In Africa 5 of the wides occur all over the continent, 9 in three out of the four divisions, 8 in two divisions and 3 in one division only.

The African endemics include 3 out of the 5 species peculiar to Jebel Marra; and for the rest, 14 are N.E. and tropical, 11 are N.E., 5 are N.E., tropical and south, while 4 are tropical.

The middle zone. 27 of the 52 species are confined to Africa; 25 are wides. Of these latter 13 are wide tropical or cosmopolitan, 3 are north temperate, 5 are African-Indian and 4 are Mediterranean. In Africa 8 of these occur all over the continent, 9 in three divisions, 6 in two divisions and 2 in one division (N.E.).

The 27 African endemics include the two remaining Jebel Marra endemics, while for the rest 11 are N.E. and tropical, 7 are N.E., 4 are N.E., tropical and south, while 3 are tropical.

The lower zone. 16 of the 22 species in this zone are confined to Africa; 6 are wides. Of these latter 3 are Old World tropical, 1 is wide tropical, 1 is north temperate and 1 is African-Indian. In Africa 2 occur all over the continent and 4 are N.E., tropical and south.

The 16 African endemics are divided into 9 N.E. and tropical, 4 tropical, 2 N.E., tropical and south, and 1 is N.E. only.

In general the zonal treatment of the flora shows a negative result and it is clear that such a treatment does not in any way serve to separate the flora into its constituent elements. On the other hand, certain minor points of interest are apparent of which the following are the most important:

1. The zones decrease in richness of species from above downwards.
2. The proportion of African endemics increases from above downwards.
3. The African-Indian species are most characteristic of the upper and middle zones.
4. The wides of the lower zone are more widely distributed in Africa than are those of the upper and middle zones.
5. Northern temperate species occur in all three zones.
6. Mediterranean species occur chiefly in the upper and middle zones.
7. The tropical African element is strongest in the lower zone.
8. The converse of 2. The wides are most noticeable in the upper and middle zones.

Comparison with Floras of other African Mountains

An interesting comparison is that of the flora of Jebel Marra with the floras of the three great mountain masses in equatorial Africa, *i.e.* the eastern group including Kilimandjaro, Kenya and Elgon; the central group of the Ruwenzori mountains and the western group of the Cameroon mountains.

16 of the species found on Jebel Marra are also to be found on all the other three groups. Most of these are either very common tropical plants or common northern temperate plants widely distributed on tropical mountains. Only three, *Asparagus africanus*,

Maesa lanceolata and *Ranunculus pinnatus*, are African endemics and these are widely spread within that continent.

11 species are common to Jebel Marra, Ruwenzori and the Cameroons. These again are mostly widespread species, but in this case the northern temperate element is absent.

6 species are common to Jebel Marra, Ruwenzori and the Kilimandjaro Mts. These species are for the most part African endemics and do not call for special notice.

11 species are common to Jebel Marra, the Cameroons and Kilimandjaro Mts. The northern temperate element is absent here, the species being either widespread tropical plants or African endemics. *Blaeria spicata*, a plant characteristic of the mountains of Africa, may be mentioned.

5 species are common to Jebel Marra and Ruwenzori only, 4 being African endemics and 1 a common tropical plant.

7 species are common to Jebel Marra and the Kilimandjaro group only. 5 of these are African endemics, 1 is a cosmopolitan species (*Chenopodium murale*) and 1 (*Epilobium hirsutum*) is a northern temperate plant with a very close relative in S. Africa.

30 species are common to Jebel Marra and the Cameroons only —a strikingly high number. 17 are African endemics. 2 are more or less cosmopolitan and characteristically northern temperate (*Lotus corniculatus* and *Nasturtium officinale*) and 4 are wide tropical forms. The remaining 7 species have a distribution extending across Asia and in nearly all cases to India.

The actual relationships of these various floras can also be expressed by the number of species which Jebel Marra has in common with each of the others. Thus 69, or 48·5 per cent., of the former flora occur also on the Cameroon Mts., 41, or 28 per cent., are found on the Kilimandjaro group, while 38, or 26 per cent., are found on the Ruwenzori Mts. Hence the relationship is very much the closest between Jebel Marra and the Cameroons although these mountains lie at a much greater distance than do the others. (Admiral Lynes informs me that the reverse is true of the birds.)

SUMMARY

Taking into account the size of Jebel Marra and its geographical position, the flora is small. This is correlated with the nature of the terrain, which is extremely barren. Not only is the flora small but it is also very generalised. 53 families are represented, all very widespread families, mostly with a strong development in the northern

hemisphere. The average size, in genera, of the families represented is very much above the average for families throughout the whole world. The genera number 127 and these again are nearly all widespread genera, the great majority having a distribution over at least one-quarter of the world's surface. Another feature is the number of large genera present, no less than 84 per cent. being above the average size. The actual average size of the genera represented is 150 compared with 14 as the average size of genera throughout the world. The species number 145. Five of these form the endemic element in the flora; all are closely related to more widely spread tropical African species. The remaining species fall into two classes; rather more than half are African endemics, rather less than half have a wider distribution.

The flora is seen to be composed of four elements, namely:

1. Cosmopolitan or very widely distributed species.
2. A tropical African element.
3. An African-Indian element.
4. A Mediterranean-West Asian element.

These elements are here tabulated in order of magnitude.

When the flora is compared with those of the neighbouring regions it is seen that the greatest degree of relationship is with that of N.E. Africa, here comprising Egypt, the Nile land and Abyssinia. No less than 127 of the species on Jebel Marra are found also in this region. The next close relationship is with the flora of tropical Africa, 107 species being common to both. 101 of the plants of Jebel Marra are actually to be found on the Abyssinian Mts. Very little relationship is revealed with N.W. Africa, and the 26 species which are common to both floras are all very widespread forms.

A zonal analysis of the flora shows that the various elements are equally scattered over all three zones and are in no way segregated. Finally a comparison may be made between the flora of Jebel Marra and those of the three great groups of equatorial mountains. This shows that by far the closest degree of relationship is with the flora of the Cameroon Mts. This again serves to emphasise the presence, in the Jebel Marra flora, of an African-Indian element.

Conclusions

It has been stated above that nearly all the plants on Jebel Marra are representatives of very widely spread and unusually large genera and families. Further, it is noteworthy that, considering

the number of species, a very large number of genera and families are represented. The endemic element also is small, not only in actual numbers but also in that the species are all closely allied to others of the same genera. In fact the degree of peculiarity in the flora is very slight. Another important feature is that in nearly all cases Jebel Marra is on the edge of the individual distributional areas of the various units composing its flora—it is not a centre of distribution in any sense.

These facts give great support to the view that the flora is a derived flora and has not developed *in situ*. This view is in complete accordance with what little geological evidence is available. Earlier in this paper Jebel Marra has been described as a volcanic massif rising out of a plateau of metamorphic rocks. It has been formed, presumably, by the volcanoes bursting through the plateau. The latter is of very great geological age and need not be further considered, but there seems to be every reason to believe that the volcanic portion is of Tertiary age though whether early or late Tertiary cannot be determined at present. As far as we know, an Angiospermous flora of a modern type had by this time become widely spread and well established over the earth's surface. The effect of the volcanic activity must have been to elevate a great mass of material which sooner or later became a virgin soil suitable for plant colonisation from the surrounding floras.

In the summary given above four floral elements are distinguished as constituting the present flora of Jebel Marra, and each of these requires to be discussed shortly. All floras exhibit what may be termed a probability element. For example the flora of any given spot (outside the Polar circles) may be expected to contain a certain number of those plants which have a cosmopolitan distribution. Similarly the flora of a spot within the tropics will contain some of the plants which have a pan-tropical distribution. In the case of a spot in tropical Africa two other groups of species will be present, *i.e.* those widely diffused through the Tropics of the Old World, and those distributed through tropical Africa itself. This probability element comprises a very large proportion of the flora in the case of Jebel Marra, and the main cause of the presence of these plants is merely the geographical situation of the locality. All the tropical African species do not, however, fall into this category, since it only includes those with a distribution throughout the tropics of that continent. The remainder will be referred to again below.

Included in the first of the four elements of the flora is a group

of species characteristic of the northern temperate regions. For the most part these are also to be found on the equatorial African mountains but they are not truly cosmopolitan and their occurrences in the African mountains are markedly isolated from their northern distributional areas. It is therefore suggestive that these plants reached Africa under conditions very different from those prevailing now. Perhaps the most facile explanation of their presence is that they are relics of the time when the present northern temperate flora occupied, under stress of glacial conditions, a zone much further south. They may either have reached Africa during the southward movement of the flora to which they belong or they may have been left behind at the higher levels when the retreat of the ice caused an earlier African flora to migrate northwards. In this case a movement northward would be replaced by a movement upwards towards higher altitudes.

Another element which requires explanation is that of the African-Indian genera and species. These have a distribution in a direction east and west from India through central Asia and Arabia to the Cameroons. Many more of these plants have a distribution from India to varying distances westwards than have a distribution from Africa eastwards and so it would appear that the direction of movement has been westwards from an eastern Asiatic centre. Whatever the explanation of this may be it is at least significant that such a path and direction has been present and open throughout a long period of geological time.

A fourth element is the Mediterranean. The plants included therein have mostly a distribution through Egypt and Syria into western Asia. The same hypothesis of glacial migration will explain these. They may even be a part of the African-Indian element which has lost touch with eastern Asia or has been carried out of that region by migration.

These suggestions as to the origins of various parts of the flora of Jebel Marra also help to explain the case of those tropical African species which have so far not been considered. The retreat of the Pleistocene Ice took place comparatively recently and there is reason to suppose that the whole process has not yet been completed. If this is so it is evident that the present tropical African flora is spreading northwards and that those species occurring on Jebel Marra are the advance guards of larger numbers. It is noteworthy that in many cases Jebel Marra lies towards the northern edge of the distributional areas of the plants concerned.

There is a group of African species on Jebel Marra which are not strictly tropical and occur only in the north-east portion of the continent. These may well be representatives of an endemic flora in that region, but it is also possible that they are very narrowly distributed portions of the Mediterranean and African-Indian elements.

A very striking feature in the flora of Jebel Marra as a whole is the very slight relationship it exhibits to the flora of N.W. Africa. It is true that there are about 25 species common to both, but these are all widespread plants not specially characteristic of either region. Only one species found on Jebel Marra is a truly N.W. African form. In connection with the migration of plants during the glacial period it is generally thought that the N. African desert region formed a barrier to direct movement southwards. If this is so then the absence of N.W. African plants on Jebel Marra is but to be expected. Certainly the flora affords no evidence of any migrationary path in that direction. From its geographical affinities it seems possible that the N.W. African flora is of fairly recent development and origin from a S. European and Oriental flora. Under such circumstances it is likely that the absence of such plants from Jebel Marra is due to the fact that they have not as yet reached a latitude so far south, notwithstanding the presence of the trans-Saharan mountain system. Taking this into account, the occurrence of the single N.W. African plant on Jebel Marra is most significant and it may be predicted that the flora of such regions as the Tibesti will, when better known, be found to contain a greater proportion of these forms.

Not only does the suggestion of migrations resultant upon the glacial period explain the presence and constitution of the flora of Jebel Marra, but it also throws some light on the comparative ages of the various elements. Arguing from it, it would appear that the oldest element is that of the northern temperate plants; that the African-Indian element arrived subsequently and that the tropical African element is the most recent. The future may well find a N.W. African element establishing itself.

Finally it must be borne in mind that quite probably a small number of the species owe their presence in the flora to accident or to individual peculiarities, but such can scarcely compose any large proportion of the flora.

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AN IRREGULAR METHOD OF POLLEN FORMATION IN *SOLANDRA GRANDIFLORA* Sw.

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(With 19 figures in the text)

1. Introduction

While making a chromosomal survey of the Solanaceae the writer had occasion to examine the pollen mother-cells of *Solandra grandiflora*. A preliminary investigation showed cytological conditions of peculiar interest, and it was decided to make a more detailed study of the irregularities occurring during the heterotypical and homotypical mitoses and the subsequent unusual nature of the pollen-formation.

2. Material, Methods

Material was obtained from the Botanic Garden, Cambridge, and the writer is indebted to the Director for generous supplies. As far as the horticultural history of *Solandra grandiflora* can be investigated there is no reason to suppose that the species in the Garden is anything but a so-called "good species." It is worth while noting in the light of the discussion at the end of this paper that the plant is recorded to have set good seed over a number of years. In March, 1923, stages in pollen formation were obtained while the plant was under excellent environmental conditions; in February, 1924, the observations recorded were in all respects similar, although the plant had been transplanted and was in a state of arrested vigour.

Belling's aceto-carmine method (1) was used, and this, with certain modifications, and a suitable dilution technique gave excellent results, the chromosomes staining black while the cytoplasm remained practically colourless. Figures are drawn from aceto-carmine preparations, with the aid of a camera lucida.

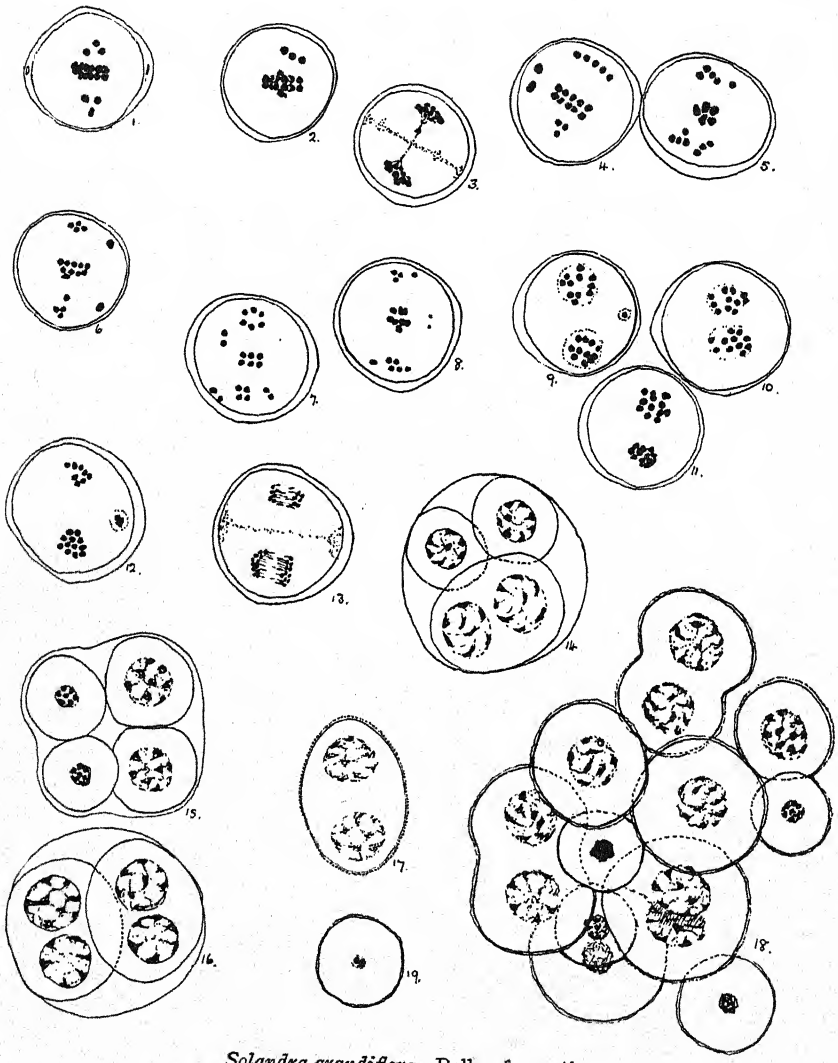
3. Pollen Formation

The early stages in meiosis are normal and will not be described in detail here. During the reduction division, however, and more particularly during the anaphase of the heterotype division, there are striking deviations from normal behaviour. For this reason it was difficult to decide upon the number of chromosomes characteristic of the species. Counts were attempted on many pollen mother-

cells at different stages in heterotype and homotype division with the result that 11 was selected as the probable, 12 as a possible, haploid number of chromosomes. The anthers are over an inch long and the mother-cells very minute, hence there are many thousands of pollen mother-cells in each loculus, yet in all the stamens examined only two or three instances of "normal" behaviour are recorded. Figs. 1–8 show characteristic distribution of chromosomes during anaphase I. It often happens that two or three chromosomes arrive at the poles some time before the rest of the chromosomes have left the equatorial plate (Fig. 1). Sometimes, however, this only occurs at one pole (Fig. 2). "Lag" (Fig. 3) is not common: it may or may not be accompanied by other irregularities. The state of affairs shown in Fig. 7 is perhaps the most general; chromosomes arrange themselves in groups of two or three or more throughout the cell. All of them appear to be "reduced" but seem to have escaped from the ordinary control exercised in spindle formation. Occasionally one or two chromosomes larger than the rest and therefore presumed to be "unreduced" are cast into the cytoplasm and take up a position on the periphery of the cell (Figs. 4, 6). These persist and can often be traced in the homotype division (Fig. 12). Other chromosomes pass into the cytoplasm and perish there: they can be seen in various stages of degeneration, but may persist as minute specks of chromatin during tetrad-formation. It may be significant that these extruded chromosomes generally appear in pairs, but there is no evidence to show that they represent a pair of homologous chromosomes. Thus there are here two forms of "chromosome diminution," in one of which the chromosomes persist and eventually form accessory nuclei, and in the other perish in the cytoplasm.

In most cases the majority of chromosomes eventually arrive at the poles and are reconstituted to form nuclei. It is evident, however, that the chromatinic content of the nuclei at the two poles may be quantitatively different. Sometimes 11, 12, or even a higher number of chromosomes will arrive at one pole, while the other has a smaller number, 5, 6 or less. Counts made of homotypical equatorial plates frequently show an inequality in number at either pole (Fig. 12). This undoubtedly gives rise to the peculiar type of pollen formation characteristic of the species, since a tetrad is commonly found to consist of two large and two smaller grains, the members of each pair approximately the same size (Fig. 15). The homotype division is more regular; Fig. 13 shows a typical anaphase, in which one spindle contains the normal number of chromosomes, while the other

has a smaller number. The protoplasm of the cell is already constricting for a division in one plane. It often happens that this is



Solandra grandiflora. Pollen formation.
Figs. 1–8: Heterotype division. Figs. 9–13: Homotype division.
Figs. 14–19: Types of pollen formation. (For explanation see text.)

the only division. At least 50 per cent. of the whole number of pollen grains are binucleate, but it would appear from a comparative study of the size and configuration of the nuclei that the binucleate con-

dition is only found when the chromatinic content of the individual nuclei is fairly high. When the number of chromosomes in the nucleus is small, uninucleate grains are formed. It is interesting that even these very small nuclei, constituted perhaps from one or two chromosomes extruded during heterotypical anaphase, are capable of forming an extine of similar composition to that of the larger grains (Fig. 19). Figs. 14–16 show immature grains; Fig. 17 a mature binucleate grain, and Fig. 18 a group of almost mature grains showing the binucleate condition in which an attempt at a second cleavage has been initiated, but not completed. The smaller grains are characterised by small compact nuclei, in which the chromosomes completely lose their identity. In the larger nuclei the chromosomes persist as recognisable entities. In no case is there record of accessory chromosomes or nuclei within the cytoplasm of an adult pollen grain. The functional capacity of these variable pollen grains will be discussed in the next section.

4. Discussion

Excluding well-authenticated cases of hybrid cytology, irregularities in meiosis, chromosomal diminution, supernumerary pollen grains and the like, have been observed in many plants. Reference is made to Winge (7), who gives a concise historical account of these phenomena. *Solandra grandiflora*, however, presents certain unique features which make an interesting comparison with irregularities in other forms. Winge (7*a*) makes use of the term "heterochromosomes" to describe chromosomes which play an unusual or irregular rôle in meiosis; thus he calls the extruded, heterotypically divided chromosome in *Callitriche verna*, a heterochromosome, and at the same time refers to the "accessory chromosome" (sex-chromosome) of animals under the same title, although he makes it clear that there is no exact correspondence between the two. Since it has been shown by Miss Blackburn (2) and others (7*b*) that real heterochromosomes (sex-chromosomes) do occur in plants, it is proposed to use a new term, "erratic chromosomes," for chromosomes behaving irregularly, as in *Solandra*, *Callitriche* (7), *Agave* (5), *Hemerocallis* (4), etc.

The so-called heterochromosomes of *Callitriche verna* behave in some ways like the erratics of *Solandra*. Winge (7) notes particularly that irregularity is commonest in the heterotypical anaphases and that the chromosome complements at the two poles are frequently unequal. Unlike *Solandra*, however, the extruded chromosomes, which are generally in the form of split or united *pairs*, persist

throughout both divisions and are often present in the cytoplasm of adult pollen grains. In *Callitriche* hexads of pollen grains, varying greatly in appearance and nuclear constitution, are the rule, in contrast to the tetrads, triads and diads of *Solandra*. Binucleate grains of the type described in *Solandra* have not, to the writer's knowledge, been described before. In both species small cells with low chromatinic content and fully-formed extine maintain themselves as pollen grains.

The question of the functional capacity of such abnormal pollen grains has been the subject of discussion for some time. The consensus of opinion is still against the possibility of normal fertilisation by their means, although it is allowed that apparently normal germ-cells can be found which lack the full complement of chromosomes characteristic of the species.

On the other hand, during the whole of this investigation, in the course of which many hundreds of pollen grains came under survey, few cases of normal tetrad-formation were recorded, and only two or three examples of normal heterotype and homotype divisions were observed. There is no reason to suppose that *Solandra* is sexually impotent; there is little, if any, pollen degeneration; successful hand pollination was carried out in 1923; and fertile seeds are known to have been set for many years. It will be of interest to study cytological conditions in the embryo-sac of *Solandra*; although a preliminary investigation has shown no signs of irregularity. It is true that irregularities are nearly always found in sexually abnormal forms—hybrids and apogamous species (3), but the frequent and regular occurrence of erratic chromosomes and other deviations from normal behaviour in so-called "good species" is a matter that calls for further investigation. Hybridisation in the more or less remote past is always a possibility, but it is improbable that this will be an explanation in every case. It is also unlikely, and in *Solandra* the possibility has been excluded, that weather or other environmental conditions are responsible for such unusual behaviour.

5. SUMMARY

1. Meiotic divisions in the pollen mother-cells of *Solandra grandiflora* Sw. are described in which persistent irregularities of behaviour are observed.

2. The number of chromosomes characteristic of the species is probably 11 (possibly 12).

3. Irregularities are first observed in the anaphase of the hetero-

type division when an autoregulative discarding of chromosomes takes place.

4. Chromosomal diminution may take place in two ways; chromosomes may be extruded into the cytoplasm and apparently perish there, or they may persist and ultimately form pollen grains.

5. At the end of the heterotype division the nuclei at the poles frequently show a quantitative difference in their chromosomal constitution.

6. In this way the homotype division often leads to the production of two pairs of pollen grains, one small and one larger pair.

7. Pollen grains may be the result of diad-, triad- or tetrad-formation. They are very variable in appearance owing to the differences in their chromatinic content. Binucleate grains, due to the absence or incomplete operation of the second cell cleavage are common. Grains with a very small chromatinic content form a characteristic extine and maintain themselves as separate entities. Very few normal tetrads are recorded.

8. To avoid confusion in terminology the name "erratic chromosome" is proposed for any chromosome which escapes or is thrust out from the sphere of action of the spindle. The term heterochromosome is reserved for the sex-chromosome.

9. It is concluded that irregularities in meiosis in *Solandra grandiflora* are of normal occurrence and are not due to weather or other environmental conditions. Nor are they due, as far as can be ascertained, to any hybridity in the history of the species.

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THE VASCULAR PLANTS CHARACTERISTIC OF PEAT

A CRITICISM

IN the July number of *The Naturalist*, Hinchliff and Priestley (1) publish, under the above title, some interesting observations on the fat metabolism of peat plants in relation to their moorland habitat. In the same paper they give an account of certain culture experiments on *Calluna vulgaris* designed to determine the effect upon growth of the calcium content of nutrient solutions used for sand-cultures. To the writer, working with experimental cultures of *Calluna* since 1911, these experiments present several features open to friendly criticism, and the conclusion drawn from them by the authors is susceptible of explanation other than—or additional to—that offered.

Attention may be directed to the following points:

1. *The general growth of the cultures.*

Sand cultures of germinating seeds and of seedlings "transplanted from deep moorland soil" were carried on for eight months from June to February, 1923, *i.e.* during a period including practically the whole active vegetative season of the plant.

It is not stated whether the two sets of cultures behaved differently, nor is any comparison made with the growth of control plants in moorland peat during the same period: all cultures are apparently included under the general statement—"during which time (*i.e.* June to February) very little growth in length of shoot occurred."

In the writer's experience, it is extremely difficult to transplant successfully seedlings of the size described from soil to sand, or indeed to transplant successfully at all during the period of active growth if the roots are completely washed out. The production of "wiry twisted and matted roots" is quite characteristic of such plants, and it would be of interest to know whether the roots of transplanted seedlings and of those germinated *in situ* showed identical features, and also whether roots, as compared with shoots, exhibited active growth during the period that the cultures were carried on. It may be noted that the final condition of the experimental plants is recorded as "relatively healthy," "unhealthy" and "dead."

At various times vigorous well-rooted plants of *Calluna* have been grown in water-cultures, and in general, although much more difficult

to manipulate, it has been found that these yielded better results than sand cultures. Sand and water cultures have been carried out designed to estimate the effect on growth of calcium salts, of different *p*H values in the solutions, and of alterations in the ratios of metallic ions present. Owing largely to the fact that *Calluna* and allied ericaceous species are unfavourable subjects for this kind of experimental culture, and hence, that the effect of generally unfavourable root environment must be carefully discriminated from specific effects —such as that due to differences in the amounts of calcium—the results hitherto obtained have not been regarded as sufficiently decisive for publication. With a technique improved by experience, it is hoped that a series of cultures now in progress may yield data of a more convincing kind.

In the experiments described by Hinchliff and Priestley, the number of experimental plants used, viz. 4 to 10 per culture solution, is regarded as too small to provide satisfactory evidence.

2. *The culture solutions.*

The six experimental solutions used all contained the following salts: $MgSO_4$, KH_2PO_4, $NaHCO_3$ and $Ca(NO_3)_2$.

The method adopted was to add a varying number of cubic centimetres of a molecular solution of each of these salts to each litre of solution made up with distilled water. Thus, $MgSO_4$ was present either as 18 c.c. or 9 c.c. per litre, KH_2PO_4 as 15 c.c. or 7·5 c.c. per litre, $NaHCO_3$ as 10 c.c. or 5 c.c. per litre, while the amount of $Ca(NO_3)_2$ varied from 2 c.c. to 20 c.c. per litre. It will be noted that the proportions of Ca ions vary directly with the nitrate content of the solutions.

The behaviour of seedlings watered with these solutions is assumed to depend directly upon the proportion of calcium present, other factors being ignored, and the general conclusion reached that,— "solutions containing low proportions of calcium salts were more favourable to the growth of *Calluna vulgaris*, under the conditions of experiment, than were those of relatively high concentrations."

As a general statement this may be perfectly true, but it is not, in the writer's view, a conclusion which follows necessarily from the experiments described, since the constitution of the solutions introduces at least two independent factors known to produce marked effects upon growth under experimental conditions. These are, (*a*) the total concentration of salts, and (*b*) the nitrate content of the solutions.

(*a*) *Concentration of solutions.* In a paper published in 1913 by the writer mention is made of water cultures of *Calluna* carried on continuously for 12 months, and it is noted that one result of these cultures was to "confirm previous observations as to the inability of the plant to thrive in any but very weak solutions" (2). In a paper read before Section K, British Association at Dundee in 1912, it was also put on record that *Calluna* seedlings in water culture are "very sensitive to changes in the concentration of the culture fluids (3)."

Again, dealing with the "calcifuge habit" in *Calluna*, it is noted that "seedlings of *Calluna* are extremely sensitive to the concentration of the solutions. In my cultures a total concentration of 0·05 per cent. gave optimum results (4)."

The water cultures on which these observations were based were carried on from June, 1911, to January, 1912. The seedlings were placed in them while still in the cotyledon stage and many of them developed branched shoots of 2 to 3 cm. in length and root-systems extending to 5 cm. within a month or so of planting.

The solution used as a basis was as follows:

KNO_3	1·0 gm.
$MgSO_4$	0·4 ,,
$CaSO_4$	0·5 ,,
$CaH_4P_2O_8$	0·5 ,,
NaCl	0·5 ,,
FeCl	trace
Distilled water	1000 c.c.

In a preliminary series of cultures to test the effect of concentrations ranging from 0·5 per cent. to 0·05 per cent. total concentration of salts, it was found that growth was adversely affected in the higher concentrations and that optimum growth took place with dilutions giving total concentrations of 0·1 per cent. or even 0·05 per cent. of salts.

The proportions of salts in the solutions used by Hinchliff and Priestley are given in cubic centimetres per litre. If calculated it will be found that the solution of highest concentration (No. 3) has a total concentration of salts above 0·7 per cent. The experimental results cited lead the writer to believe that concentrations so high as this inhibit growth and cannot be left out of account in estimating the effect of different factors in the solutions used.

(*b*) *The nitrate content.* In a paper published in 1922 (5) an account was given of certain experimental cultures carried out during a study

of the possibility of fixation of atmospheric nitrogen by the endophyte of *Calluna*. In these cultures it was found that optimum conditions for growth of the seedlings were secured in pure culture by supplying a nutrient medium *lacking* combined nitrogen. The cultures were carried on from April to October; the seedlings made unusually healthy and vigorous growth, in every way comparing favourably with that of controls supplied with 0·5 gm. KNO_3 per litre. They were bright green in colour and exhibited none of the symptoms quickly developed by ordinary seedlings grown in solutions lacking combined nitrogen.

In view of these results and those of subsequent experiments, it is suggested that the amount of nitrate used by Hinchliff and Priestley may have had an effect upon the vigour of the plants quite independently of calcium content.

In one case as much as 20 c.c. molecular solution of $Ca(NO_3)_2$ per litre was used—a concentration exceeding 0·2 per cent., and therefore near the limits of *total* concentration at which a deleterious effect upon growth was observable in the water-cultures just cited. It is significant that the solution most rapidly toxic (No. 3) was that of highest total concentration containing the maximum quantity of nitrate, and also that the seedlings in this solution showed a "conspicuous growth of fungus hyphae" towards the close of the experiment. The least toxic solutions (Nos. 4 and 5), supplied to plants recorded as still "relatively healthy" eight months after the start of the experiment, are also those of lowest total concentration—0·26 per cent. and 0·3 per cent. respectively. It is admitted that these criticisms do not explain the relative toxicity of solution No. 6 of low concentration as compared with No. 1 though containing more calcium and, incidentally, more nitrate. It may be pointed out that the nutrient solution mentioned above and habitually used by the writer for culturing *Calluna* contains two calcium salts added in quantities equivalent to almost 6 c.c. of molecular solutions per litre (*loc. cit.* (4), p. 27).

With regard to these cultures as a whole, it is felt that more convincing evidence as to the effect of calcium on growth could have been deduced by the use of solutions uniform as to total concentration of salts and concentration of nitrates, *i.e.* in which amount of calcium is more nearly the only effective variable.

Passing to the very interesting observations on accumulation of fats in the tissues of *Calluna* seedlings raised under aseptic conditions from sterilised seeds, it may be pointed out that the seedling

figured was several months old and had been exposed to light from germination.

If, as appears likely, oil is not only stored as a reserve in seeds but is formed regularly during metabolism, the inhibition of growth and development associated with the absence of specific fungal infection might be expected to lead to a "glut" of oil in some part of the tissues as a consequence of translocation during germination and (or) of photosynthesis subsequently.

What the exact relation is between inhibition of growth, accumulation of fatty material in the tissues, and metabolic activity of the endophyte has yet to be learned. It is hoped that a comparative study of the metabolism of the endophyte in pure culture on various nutrients, including *Calluna* oil extracted from seed and kindly supplied recently by Prof. Priestley, may contribute some positive data bearing on the problem.

In a paper now in the press the necessity of distinguishing between *infection* and *mycorrhiza formation* in *Calluna* has been stressed by the writer. The latter part of the statement on p. 208 of the paper now under consideration—that certain "experiments show that *Calluna* seedlings are unable to grow in pure culture free from mycorrhiza" should read "free from infection by the mycorrhizal fungus."

The two phenomena are distinct and no experimental evidence has been offered hitherto by the present writer that the formation of mycorrhiza is obligate in *Calluna*, although under normal conditions it follows upon infection of the seedling tissues. Recent work has emphasised the importance of this distinction.

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REVIEWS

General Botany and the Biology of Plants

General Botany, an Introductory Text for Colleges and Advanced Classes in Secondary Schools, by E. N. Transeau. New World Science Series, World Book Company, Yonkers-on-Hudson, N.Y. 1923. Pp. x and 550, with 351 illustrations.

Laboratory and Field Work in General Botany, by E. N. Transeau and H. C. Sampson. Same publishers, 1924. Pp. vi and 154.

Dr Transeau, who is professor at the University of Ohio, states in his preface that the works here noticed are the outcome of a rather wide experience of teaching, and especially of seven years at his present university. Four sources of the material and ideas presented are mentioned: (1) the traditional course in general botany, embodying the facts and principles which most botanists agree are essential for a foundation in the subject; (2) subject-matter derived from the suggestions of the large body of men engaged in the teaching or practice of horticulture, agriculture and forestry; (3) information from the students to whom the course has been given, their relative interest in different kinds of subject-matter and responses to different modes of presentation; (4) questions asked by the public—often unanswerable, but always suggestive. This enumeration makes clear the author's desire to teach botany to his students with the broadest possible outlook on practical life. In the practical work he tries to use "field and laboratory time for the answering of questions and the solving of problems rather than for the making of detailed drawings," and this, he says, "has changed the attitude of our students toward laboratory work" and "has also made it possible to cover a far greater range of materials and principles than formerly."

One can imagine a progressive but cautious teacher assailed by grave doubts in reading Dr Transeau's preface, and wondering if students so taught would not lose in thoroughness more than they gained in range and breadth. It is true that the final test can only lie in the future work of the students, but it is of interest to see how Dr Transeau actually tackles his material.

At the outset he points out the complete dependence of man on plants and their activity, and then goes on to consider the evidence that plants are living things, what is meant by environment and how it affects plants and determines the distribution of different kinds, the conception of limiting factors, and, in a very short sketch, the cellular structure of plants. The author then proceeds to deal with the different organs of a higher plant, beginning with the form and structure of leaves (not disdaining the technical names for their shapes and margins) and proceeding with a chapter on their fundamental work in the manufacture of food. In this he brings out very clearly and with the aid of simple diagrams the genetic relations of the sugars and the other carbohydrates to fats and proteins, and a chapter is devoted to the substances made from foods manufactured by plants which are of use to man. Following chapters on the light and water relations of leaves there is a chapter on the physical processes involved in the movements of substances within plants, one on the water balance, and on the growth and fall of leaves. The stem and the root are next dealt with in a similar manner, and then follows an account of the environmental factors affecting growth and reproduction, in which the work of Garner and Allard on the striking effects of different lengths of day is dealt with, as well as the seasonal influences, water and temperature relations, and such subjects as the carbohydrate-nitrogen balance.

The subject of reproduction is introduced by a general chapter on vegetative multiplication and plant propagation, in which full use is made of illustrations

from horticultural and agricultural practice. The inflorescence and flower, sexual reproduction in the flowering plants, fruits and seeds, are next dealt with, and then a chapter is devoted to dormancy and germination of seeds. This is followed by chapters on plant breeding, variations and mutations and on hybridisation and selection, in which a clear elementary account is given of the Mendelian principle and its cytological foundation

The study of vegetation and its applications are represented by four chapters on the distribution of plants in nature, the vegetation of North America, the relation of plant industries to climatic plant formations, and weeds and their control. This section is particularly good, as might be expected from the author's own work.

After a general chapter on non-green plants the bacteria are dealt with, a special chapter being given to soil-bacteria and the nitrogen-cycle, and then fungi and plant diseases.

A short chapter on classification is followed by chapters on the different great groups, beginning with the algæ. These chapters together include about 100 pages or less than one-fifth of the whole book. A final chapter is devoted to evolution.

The treatment throughout is clear and simple, and of course very elementary. Its interest is somewhat unequal, and if a general criticism may be made it is that the author would have done better to have treated the more difficult and doubtful points raised rather more fully and with a somewhat more explicit recognition of their difficulty. This applies perhaps most particularly to heredity, variation and evolution. The great positive merit of the book lies in the large numbers of facts derived from common observation and from the experience of plant industries which are interwoven with the general exposition. The *range* of phenomena dealt with is perhaps somewhat too great for adequate treatment (we mean of course adequate from the point of view of the elementary student), but there can be no doubt of the primary interest of a very large proportion of the topics unusual in an elementary course with which Prof. Transeau deals, and of his skill and ability in the treatment of most of them.

As the author says in his preface, the efficient use of the time given to field and laboratory exercises is equally important with selection of subject-matter, for the practical work is, after all, the heart of the course. The laboratory and field work follows the order of treatment adopted in *General Botany*. The field work appears to occupy about one-third—a far larger proportion than is usual—and the laboratory work about two-thirds of the whole, the two being interspersed, while three or four periods are given to greenhouse and library work. There does not appear to be any garden work, an omission which is rather surprising. The directions for practical work appear under 45 heads and include instructions for observation and experiments, and questions. The records are intended to take the form of notes and diagrams, rarely of drawings. The importance of drawing in rendering observation close and accurate is, in the reviewer's opinion, underrated. One doubts if the results of many of the observations asked for would be sufficiently impressed on the student to be of much future use. It is also not quite clear from the instructions whether certain of the questions are supposed to be answered from observation or from the results of instruction. It is indeed not easy to get a clear picture of exactly how such a course of instruction as that represented would work out in actual practice, so that intelligent criticism is difficult.

But it may be said with confidence that a student following this course would undoubtedly obtain both a far wider knowledge and a far truer perspective of the science of plants as a whole and of its importance to man than he would from most of the courses given in this country. Whether he would get so deep or so trustworthy a knowledge of particular parts of the science is more difficult to decide. It may very well be that the more vivid interest aroused and the training in initiative and thinking for himself more than compensates for any deficiency in thoroughness. A. G. T.

The Biology of Flowering Plants, by MACGREGOR SKENE. London, Sidgwick and Jackson, Ltd., 1924. Pp. xi and 523, with 8 plates and 68 figures in the text. Price 16s. net.

It is a pleasure to welcome this excellent book on a branch of botany which has on the whole been a good deal neglected in our text-books of recent years. The term "biology" for the vital relations of plants to their environment, commonly used for many years, especially in German, has been objected to on the ground that the word should be reserved for the science of life as a whole. But the objection is perhaps rather pedantic, because no real confusion arises and no very satisfactory substitute has been devised. "Ecology" in its narrower meaning of the science of the habitat is something distinctly different, and in its wider meaning is too wide. "Autecology" is more nearly equivalent, but has not been very much used, and "bionomics" also has not succeeded in establishing itself, at least in botanical terminology.

Dr Skene's book is soundly based on physiology, but always deals with plants in nature, and is by no means confined to their physiological relations in the narrow sense, for though a large proportion is occupied with photosynthesis and the water relation, the book includes fairly detailed discussions of such subjects as the relations of insect visitors to flowers, in which connexion the recent important work of Frisch and of Knoll receive attention. "The difficulty of giving enough physiology to make the foundation sound, and yet not so much as to obscure the picture has been fully realised," writes the author in his preface, and most readers, we think, will gladly testify that he has most skilfully and judiciously hit the happy mean. So excellent a sense of proportion and so sane and critical a judgment are displayed by the author throughout the work that it is difficult to criticise or to single out any section for special commendation. All candidates for degrees in botany should make a point of carefully reading the book, and many general teachers of botany will find that Dr Skene not only refreshes their old knowledge but amplifies and brings it up to date. There is a very useful list of literature containing over 600 titles.

Experimental Pollination: an outline of the ecology of flowers and insects, by F. E. CLEMENTS and FRANCES L. LONG. Carnegie Institution of Washington, 1923. No. 336. Pp. vii and 274. 17 plates (one coloured).

This book contains a full record of very detailed and elaborate observations on the insect visitors of 17 species of wild flowers, carried out at the Alpine Laboratory, above Manitou in the Rocky Mountains of Colorado. The numbers of insects belonging to different species visiting normal flowers of each species of plant, and the behaviour of the different visitors were recorded; and various experiments such as mutilating, painting and changing the position of the flowers were made. The aim was to obtain quantitative results of the normal phenomena of visitation by different insects, and also to evaluate the factors of initial instinctive response of the insects to the different stimuli presented by the flowers, such as conspicuousness, colour, scent, etc., and to separate these from the effects of habit, which is very marked in mature insects. Enclosing the observer with nests of insects on the point of emergence in a cage several metres long, which could be placed in vegetation containing the flowers the visits to which it was decided to observe, is suggested as a useful method. But it is pointed out that to obtain perfectly complete and trustworthy results it is necessary to deal with individual insects which have to be caught and marked, preferably on emergence from the nest.

The wide variation in the habits and responses of different genera of insects was very marked. On the whole, artificial flowers were relatively little visited, though this was by no means true of all individual cases. Painted flowers were

much more visited than the paper ones in comparison with the normal. The insects, largely bees, were often disturbed and upset by mutilations and artificially produced changes of form and could adapt themselves but little to conditions quite foreign to their experience. Honey as such showed practically no attractiveness to bees, which seem to have a very poor power of distinguishing it by smell. Strong odours from masses of flowers are apparently very effective in attracting insects from a distance, colour masses at somewhat closer range, and detailed form and colour at close quarters.

A valuable feature of the book is the large section devoted to summaries of previous work, which forms a most useful compendium of information as to what has been done in the subject. The title of the book, *Experimental Pollination*, is rather misleading. There are no experiments on pollination as such, and little information as to the actual carrying-out of pollination by visiting insects, which do not of course by any means always necessarily pollinate the flowers they visit, though of some species it is stated that pollen is rubbed on the stigmatic surfaces. The book is rather a study of the conditions under which insects of various species *visit* certain species of flower, and of their general behaviour towards normal and modified flowers. A. G. T.

New Edition of "Dixon and Jameson"

The Student's Handbook of British Mosses, by H. N. Dixon and H. G. Jameson. Eastbourne (Sumfield) and London (Wheldon and Wesley), 1924. Pp. xlviii and 582, with 63 plates. Third Edition, revised and enlarged. Price in one vol. 24*s*., two vols. (plates and text separately), 28*s*.

We warmly welcome the new and improved edition of this well-known standard handbook, which has always possessed the great merit of combining ease of use by the beginner with fullness and accuracy of information. The species and varieties added to the British Flora since 1904, when the second edition appeared, have been incorporated, and the descriptions and notes brought up-to-date where correction or amplification was called for. The plates, in which every species is figured, have been entirely redrawn from nature by Mr Jameson for this edition, with many emendations and additions. They are now reproduced as half-tone blocks and show every detail essential for distinguishing the species with great clearness. The magnification of every part drawn is added, and corresponding parts of the different species are correspondingly numbered and magnified.

It would be difficult to overpraise the clearness, usefulness and scholarly thoroughness of the descriptions and notes, which make the identification of species and the discrimination of closely allied forms a delightful task, quite free from the mind-racking perplexities imposed on the student by too many writers of floras. This is altogether a model flora, and considering its contents the price is exceedingly moderate.